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

Protocol Approve Date: 12 September 2018

Certain information within this protocol has been redacted (ie, specific content is masked irreversibly from view with a black/blue bar) to protect either personally identifiable information (PPD) or company confidential information (CCI).

This may include, but is not limited to, redaction of the following:

- Named persons or organizations associated with the study.
- Proprietary information, such as scales or coding systems, which are considered confidential information under prior agreements with license holder.
- Other information as needed to protect confidentiality of Takeda or partners, personal information, or to otherwise protect the integrity of the clinical study.

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

Sponsor:	Takeda Vaccines, Inc. 40 Landsdowne Street Cambridge, MA 02139 USA
Study Identifier:	ZIK-101
IND Number:	017673 EudraCT Number: Not applicable
Vaccine Name:	• Investigational vaccine: Purified Inactivated Zika Virus Vaccine (PIZV) candidate (TAK-426) • Placebo: saline solution
Date:	12 September 2018
Version:	Protocol Amendment 3 - Version 7.0

1.0 ADMINISTRATIVE INFORMATION

1.1 Contacts

A separate contact information list will be provided to each site. Contact information is also provided in Table 1.a.

The sponsor will provide investigators with site-specific emergency medical contact information cards to be carried by each subject.

General advice on protocol procedures should be obtained through the monitor assigned to the trial site. Information on trial related responsibilities is given in Section 3.1 and relevant guidelines will be provided to the site.

Table 1.a Contact Information

Issue	Contact
Serious adverse event and pregnancy reporting	PPD Fax and telephone numbers for serious adverse event and pregnancy reporting will be provided to the site
Medical Monitor (medical advice on conduct of protocol or compound)	Emergency medical contact information will be provided to the site
Responsible Medical Officer (carries overall responsibility for the cond uct of the trial)	Emergency medical contact information will be provided to the site

1.2 Approval

REPRESENTATIVES OF TAKEDA

This trial will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical trial protocol, and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki (1).
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use E6 Good Clinical Practice: Consolidated Guideline (2).
- All applicable laws and regulations, including, but not limited to those related to data privacy and clinical trial disclosure.

SIGNATURES:

PPD

INVESTIGATOR AGREEMENT

I confirm that I have read and that I understand this protocol, the Investigator's Brochure, and any other product information provided by the sponsor. I agree to conduct this trial in accordance with the requirements of this protocol, and also protect the rights, safety, privacy, and well-being of trial subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki (1).
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use E6 Good Clinical Practice: Consolidated Guideline (2).
- All applicable laws and regulations, including, but not limited to those related to data privacy and clinical trial disclosure.
- Regulatory requirements for reporting serious adverse events defined in Section 10.4 of this protocol.
- Terms outlined in the Clinical Study Site Agreement.
- Appendix A– Responsibilities of the Investigator.

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Appendix C of this protocol.

Signature of Investigator Date

Investigator Name (print or type)

Investigator's Title

Location of Facility (City, State)

Location of Facility (Country)

1.3 Protocol Amendment 3 Summary of Changes

1.3.1 Protocol Change History

Date	Amendment Number	Amendment Type	Region
02 March 2017	Initial Protocol Version 1.0	-	Global
18 May 2017	Protocol Version 2.0	Not applicable	Global
25 August 2017	Protocol Version 3.0	Not applicable	Global
01 September 2017	Protocol Version 4.0	Not applicable	Global
27 October 2017	Protocol Amendment 1 Version 5.0	Non substantial	Global
11 December 2017	Protocol Amendment 2 Version 6.0	Substantial	Global
12 September 2018	Protocol Amendment 3 Version 7.0	Substantial	Global

1.3.2 Summary of Changes

Amendment to Protocol Version 6.0 11 December 2017

Rationale for the Amendment:

The purpose of this substantial amendment is to clarify the recording of information from physical examinations performed during the study and to extend the visit window of Visit 9 (Month 12) to allow communication with subjects who will participate in the 12/24 months follow-up phase.

The description of the interim analyses has been modified to indicate that there will be an additional interim analysis performed for the flavivirus naïve cohort only up to Visit 6. In addition, the timing of the two analyses for data accrued past Visit 6 (Day 57) has been modified.

The names of Takeda personnel have been updated and typographical errors have been corrected.

Section	Description of change
1.2	PPD

2.0	**Interim Analysis~~is~~es:** ***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). ~~This interim analysis is not intended for early termination of the trial. A second interim analysis will be performed to include the data describing the persistence of immunity at 12 months post dose 2 and safety from 6 to 12 months post dose 2.~~ **Final Analysis:** The final 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 subsequent analyses will include (1) the data up to Visit 9 (12 months post dose 2) and (2) the data up to Visit 11 (24 months post dose 2).*** 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). More details regarding the analyses will be provided in the Statistical Analysis Plan (SAP). 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 8 (Day 211, 6 months post dose 2)~~ ***to Visit 9 (Day 393, 12 months post dose 2)***, and one to report the data from ~~Visit 8 (Day 211)~~ ***Visit 9 (Day 393)*** until the end of the study, ie, Visit 11 (Day 757, 24 months post dose 2).

2.1	**Visit 7 (Day 133)** anchored to visit day Day 29 + ~~4½~~ ***3½*** mo **Visit 9 (Day 393)** acceptable visit window (days before/days after) -7/~~+14~~ ***+97*** footnote (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 ~~and~~***; findings consistent with the definition of AEs or SAEs (see Section 10.0) must be*** transcribed into the electronic Case Report Form (eCRF). For any procedures at the site, the investigator shall follow his/her standard practice. Refer to Section 9.1.4.
9.1.4	The following should be documented in the subject's source document ~~and transcribed into the eCRF~~: • The findings of complete physical examinations, • the findings of symptom-directed physical examinations, • if symptom-directed physical examinations were not required ***Findings consistent with the definition of AEs or SAEs (see Section 10.0) must be transcribed into the eCRF.***
9.1.6.2 (Table 9.a)	~~PPT (partial thromboplastin time)~~ ***PT (prothrombin time)***

13.2	***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). ~~This interim analysis is not intended for early termination of the trial. A second interim analysis will be performed to include the data describing the persistence of immunity at 12 months post dose 2 and safety from 6 to 12 months post dose 2.~~ The final 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 subsequent analyses will include (1) the data up to Visit 9 (12 months post dose 2) and (2) the data up to Visit 11 (24 months post dose 2).*** 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). More details regarding the analyses will be provided in the SAP. 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 8 (Day 211, 6 months post dose 2)~~ ***to Visit 9 (Day 393, 12 months post dose 2)***, and one to report the data from ~~Visit 8 (Day 211)~~ ***Visit 9 (Day 393)*** until the end of the study, ie, Visit 11 (Day 757, 24 months post dose 2).

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

2.0 TRIAL SUMMARY

Takeda Vaccines, Inc. 40 Landsdowne Street Cambridge, MA 02139 USA		**Product Name:** Purified Inactivated Zika Virus Vaccine (PIZV) candidate (TAK-426)
Trial 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		
IND No.: 017673		**EudraCT No.:** Not applicable
Study Identifier: ZIK-101	**Phase:** 1	**Trial Blinding Schema:** Observer-blind

Background and Rationale:

Zika virus (ZIKV) is a mosquito-borne single-stranded positive-sense ribonucleic acid (RNA) virus which belongs to the *Flaviviridae* family, genus *Flavivirus*, that presents structural similarities with other members of the *Flaviviridae*, including Spondweni virus, Yellow Fever (YF) virus, Dengue virus (DENV), West Nile virus (WNV), Japanese Encephalitis (JE) virus, and Tick-borne Encephalitis virus.

ZIKV was discovered in 1947 in Uganda while testing samples taken from sentinel rhesus monkeys used as bait during a surveillance of YF. The first human disease cases were reported in 1952 in Uganda and Tanzania. For 55 years, the virus circulated in areas of Africa, Southeast Asia, and the Pacific Islands, and was considered a rare disease with mild symptoms; only 14 human cases were reported and no outbreaks were detected during that period.

The first large outbreak of ZIKV disease was reported in Yap Island (Federal state of Micronesia) in 2007, and was followed by an outbreak in French Polynesia in 2013-2014. The first cases of ZIKV disease in South America were reported in Easter Island in 2014, and the first reports of locally transmitted infection (ie, by mosquito) came from Brazil in May 2015, which triggered the third outbreak in 2015-2016. Since then, ZIKV disease has continued spreading through the South American continent and to other regions. In the current outbreak that started in 2015, there have been estimates of more than 750,000 ZIKV-suspected cases reported from more than 60 countries/territories; most of these cases are from the South America continent.

The primary mode of transmission of ZIKV is through the bite of female mosquitoes, prominently *Aedes aegypti* and *Ae. albopictus* species that have spread globally. Vertical transmission from mother to fetus during pregnancy and the perinatal period, sexual contact, and blood transfusion are also confirmed modes of transmission of the disease. Potentially, the virus could be transmitted through laboratory exposure and by body fluid contact, eg, tears, urine, breast milk, or through solid organ transplants, but there is limited evidence to support this.

More than 80% of ZIKV infections remain asymptomatic, and for the symptomatic cases, the disease is in general mild and of short duration. Some clinical manifestations include, but are not limited to, mild fever, maculopapular rash, conjunctivitis and arthralgia.

Despite mild clinical symptoms in the pregnant woman, ZIKV infection during pregnancy has been associated with serious outcomes for the fetus and newborn. The severity of the disease is related to the consequences in the fetus and newborn child from women with ZIKV infection during pregnancy. The spectrum of congenital anomalies associated with ZIKV infection, known as Congenital Zika Syndrome (CZS), consists of severe microcephaly with partially collapsed skull, cerebral cortices with subcortical calcifications, macular scarring and focal pigmentary retinal mottling, congenital contractures, and marked early hypertonia with symptoms of extrapyramidal involvement. The exposure to ZIKV at any moment during pregnancy is a risk for fetal or postnatal developmental complications, primarily affecting brain development. Recent analysis of the U.S. Zika Pregnancy Registry (USZPR) showed that the prevalence of newborns with congenital defects from pregnancies with laboratory-confirmed ZIKV infection is 10% (95% CI: 7%-14%). This is 30 times higher than baseline prevalence before more recent Zika outbreaks.

Since the beginning of these outbreaks and up to end of September 2017, there have been 3689 confirmed CZS malformations potentially associated with ZIKV infection in 27 countries and territories of Latin America and the Caribbean.

ZIKV is a neurotropic flavivirus that can potentially cause disease within the central nervous system. Worldwide

concern over ZIKV causing Guillain-Barré syndrome (GBS) emerged when an increased number of GBS cases was temporally associated with the 2013-2014 ZIKV outbreak in French Polynesia, during which 42 GBS cases were reported, all of these cases had neutralizing antibodies against ZIKV, compared to 56% in the control group. Other neurological complications, such as encephalitis, meningoencephalitis, paresthesia, facial paralysis and myelitis, have also been associated with ZIKV infection. Based on these findings, the countries affected by ZIKV disease in the 2015-2016 outbreak improved and extended the surveillance of neurological syndromes. In 2017, after the spread of ZIKV throughout the Pacific Islands, Latin America and the Caribbean, a total of 200 cases of GBS related to ZIKV infection have been reported in these regions as case series or isolated cases. The incidences reported have increased 2 to 9.8 times compared to baseline before the epidemic in Latin America, and up to 20 times in French Polynesia. The association of GBS was only reported when the circulating strain was of Asian lineage. As of 01 February 2017, ZIKV of the Asian lineage was circulating in more than 80% of affected countries, including 48 countries in Latin America. The overall incidence of ZIKV-associated GBS is estimated to be 24 per 100,000 cases of ZIKV infection.

No specific antiviral treatment is available for ZIKV infections and no vaccine against ZIKV is currently available. As the disease is self-limiting, treatment for uncomplicated ZIKV infection is supportive and focuses on symptoms. The main recommendations to address outbreaks are through prevention (avoiding mosquito bites, reducing sexual transmission, improving the procedures for safe blood transfusion), and vector control measures.

The risk of infection with ZIKV is increasing given that the spread of ZIKV is rapid and intense in tropical and subtropical countries. Vector control is difficult to establish and maintain, and new areas continue to be colonized by the *Aedes* species. Climate changes may also create favorable environments to maintain the vector. There is an increased urban population and mobility, and the role of sexual transmission may be an important mechanism to spread rapidly the virus into susceptible populations, even in regions where the vector is uncommon.

ZIKV has posed a challenging situation for health, public and economic sectors of affected countries. The World Health Organization (WHO) declared on 01 February 2016 the Zika outbreak as a Public Health Emergency of International Concern (PHEIC) and recommended to focus the research on the causal association of ZIKV infection during pregnancy and microcephaly and other neurological abnormalities in newborns. By August 2016, the U.S. Department of Health and Human Services declared Zika a public health emergency in Puerto Rico based on the significant threat to pregnant women and their children's health. In November 2016, and after evaluating the evidence about the causal association of ZIKV infection during pregnancy and microcephaly and other neurological abnormalities in newborns, WHO announced that Zika no longer represents a PHEIC but stressed the need to escalate the disease to a sustained work program with dedicated resources to address its long-term nature and its associated consequences.

Considering the conclusive associations between ZIKV infections and fetal and postnatal abnormalities, the development of a vaccine that can provide protection is crucial for countries where the epidemic is expected to arrive and/or persist, as well as in countries in which the virus has not yet been introduced. In order to address the urgent medical need and in anticipation of possible outbreaks with rapid onsets, Takeda has initiated the development of a purified inactivated ZIKV vaccine candidate, PIZV, for use in endemic areas and non-endemic areas for prevention of ZIKV associated illness of any severity and/or infection.

The Biomedical Advanced Research and Development Authority (BARDA) from the U.S. Department of Health and Human Services' Office of the Assistant Secretary for Preparedness and Response is providing funding to Takeda for the US-based PIZV development program. Due to the need to vaccinate women and men of reproductive age, the target population of PIZV will be 9 years of age and older. In this phase 1 study, study population is limited to ≥18 to ≤49 years of age, and age expansion will be included in the next phase of development.

There are no clinical data currently available for PIZV. PIZV will be tested for nonclinical safety in a 3-dose Good Laboratory Practice-compliant, repeat-dose toxicity and local tolerance study in New Zealand White Rabbits. Toxicology data will be available prior to study start and provided in the Investigator's Brochure (IB).

ZIK-101 is a first-in-human study. It will enroll both flavivirus naïve and primed healthy adults aged ≥18 to ≤49 years. To ensure the enrollment of healthy adults, screening tests (hematology, biochemistry and urinalysis) will be performed prior to vaccine/placebo administration.

Flavivirus primed adults are included in this study to assess if there is any impact from pre-existing flavivirus antibodies. The testing of flaviviral serological status will be performed prior to randomization and will enable

stratification of randomization.

Flavivirus naïve subjects will be enrolled during the first phase of the study. If positive recommendations from an external Data Monitoring Committee (DMC) are issued following the review of safety and tolerability data in this population, when the last flavivirus naïve subject has completed study Visit 4 (28 days post dose 1), vaccination of flavivirus primed subjects will start.

Due to the ongoing threat of ZIKV disease and the urgent need of a vaccine, this phase 1 study will be accelerated to assess dose finding for PIZV, in addition to evaluation of safety and immunogenicity of the vaccine candidate. PIZV dose will be selected based on descriptive safety data in both flavivirus-naïve and primed subjects, and geometric mean titers (GMT) of neutralizing anti-ZIKV antibody levels at 28 days post dose 2 in flavivirus naïve subjects. Placebo serves as the control for the study vaccine and in the absence of effective treatment or prevention for ZIKV disease, the use of placebo in this trial is justified.

The rationale for the observer-blind approach is based on the different physical appearance of the investigational vaccine compared to the saline solution placebo that was selected and preferred to alum-containing placebo to avoid side effects associated with alum.

All enrolled subjects will receive two 0.5 mL doses of either PIZV or placebo intramuscularly (IM) on Day 1 and on Day 29. The objective of ZIK-101 is to assess the safety of PIZV and select a single vaccine dose level from three different antigen concentrations (2, 5 or 10 µg) for further development.

The primary rationale for selection of the three vaccine dose levels can be summarized as follows:

- the low-dose level (2 µg) was selected for purposes of potentially identify an immunological threshold, and was based on the limit of the analytical methods for the drug product available at the time;
- the mid-dose level (5 µg) was selected to provide a mid-point on the dose response curve. In addition, this dose level is based on the Walter Reed Army Institute of Research (WRAIR) vaccine currently tested in Phase 1, which was originally based on initial studies in non-human primates (NHPs); and
- the high-dose level (10 µg) was selected to follow an approximate 2-fold range and to further define the potential dose-response curve in both naïve and primed subjects.

The trial will be conducted in accordance with the protocol, the International Council for Harmonization of Technical Requirements for Pharmaceuticals (ICH) GCP Guidelines and applicable regulatory requirements.

Trial 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, in ZIKV endemic and non-endemic regions.

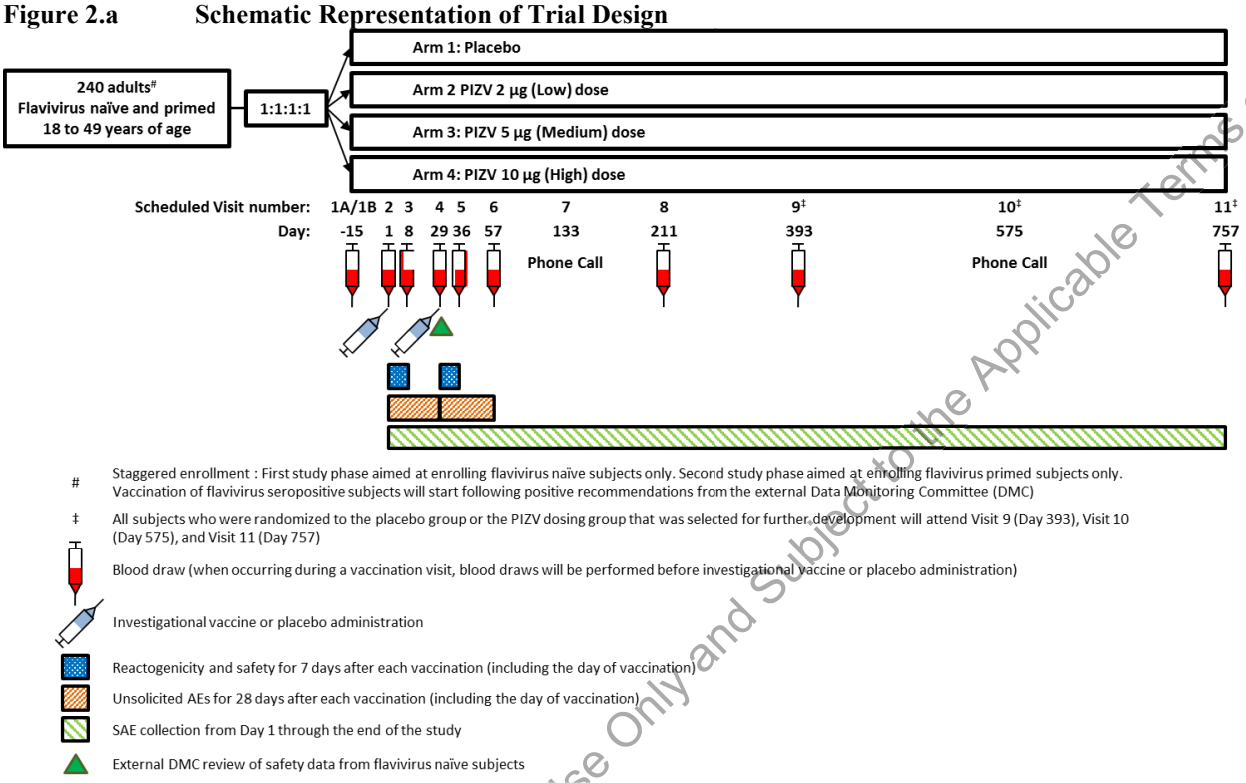
The trial will last approximately 7 or 25 months for each subject, following a screening period prior to Visit 2. The screening period will last for up to 2 weeks (14 days) prior to randomization, with the possibility that some subjects (Flavivirus primed subjects only) will need to repeat some screening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

Flavivirus naïve subjects: subjects without detectable serum antibodies against a panel of flaviviruses, as measured by a reactive antibody based assay (Luminex).

Flavivirus primed subjects: subjects with serum antibodies against a panel of flaviviruses, as measured by a reactive antibody based assay (Luminex).

Approximately 240 subjects aged ≥18 to ≤49 years will be enrolled and equally randomized into four groups (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 versus ≥30 to ≤49 years.

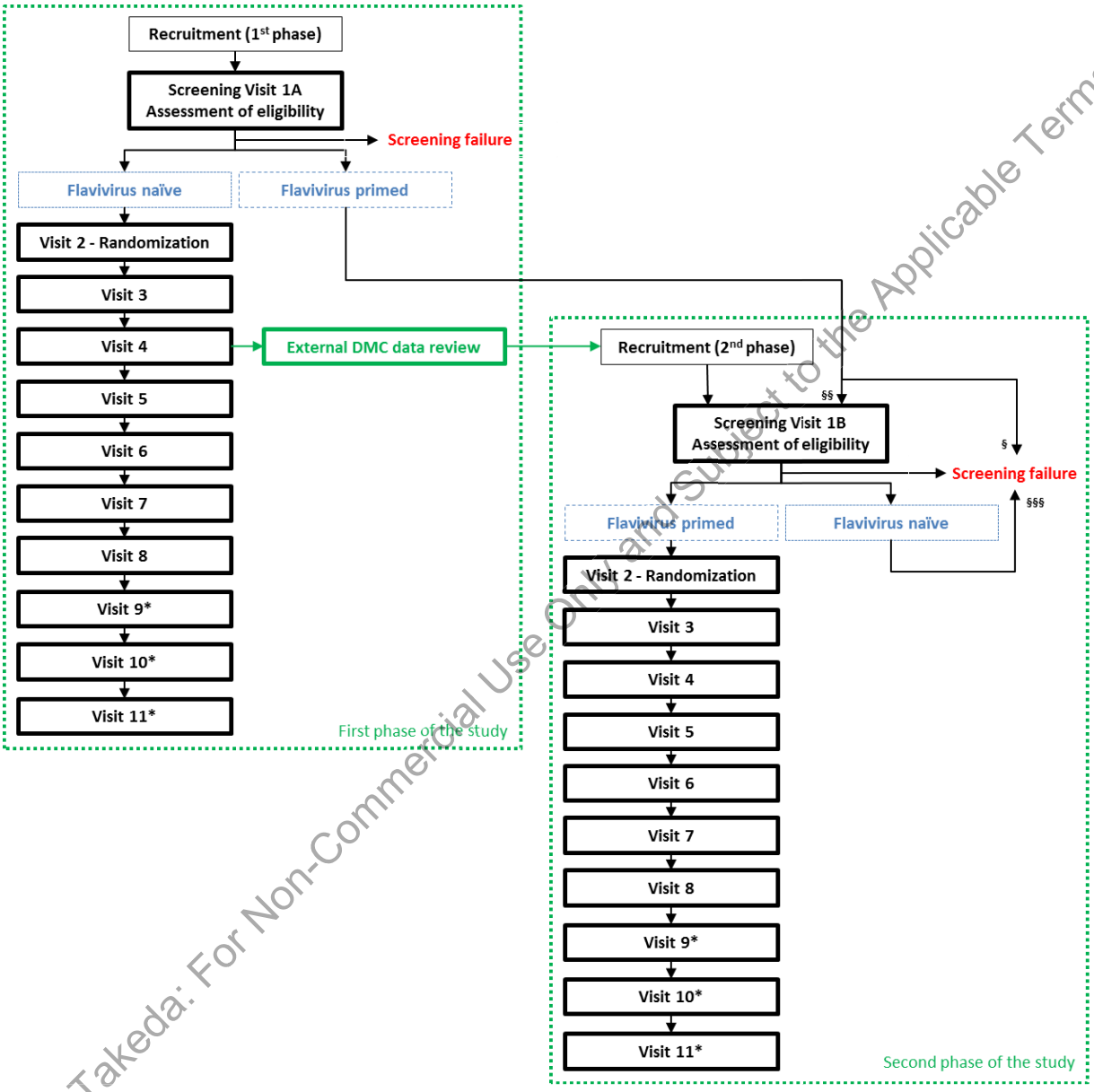
Figure 2.a Schematic Representation of Trial Design



Staggered enrollment: There will be two study phases.

- 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 (ie, 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 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 2.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 2.b §§) (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 (see Figure 2.b §§§).

Figure 2.b Staggered Enrollment



* 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
- 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, 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: ie, 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 at Visits 2 and 4 before each investigational vaccine/placebo administration for pregnancy testing.

Each subject will receive diary cards to collect solicited adverse events (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 serious adverse events (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.

Primary Objectives

- To describe the safety of two doses of 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

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

Subject Population:

Healthy subjects: Yes

Planned Age Range: ≥18 to ≤49 years

Planned Number of Subjects: approximately 240

Planned Number of Arms: 4 arms (*n*=60 per arm: approximately 30 flavivirus naïve subjects and approximately 30 flavivirus primed subjects)

- Arm 1: Placebo saline solution
- Arm 2: PIZV 2 µg (Low) dose
- Arm 3: PIZV 5 µg (Medium) dose
- Arm 4: PIZV 10 µg (High) dose

Criteria for Inclusion:

Subject eligibility is determined according to the following criteria:

- The subject is aged ≥18 to ≤49 years.
- Individuals who are in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs) and eligibility screening tests (hematology, biochemistry and urinalysis) and clinical judgment of the investigator. Vital signs must be within normal limits (ie, below Grade 1 as specified in the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers). Screening tests must be within normal limits or not be above Grade 1 as defined in the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers.
- The subject signs and dates a written, informed consent form and any required privacy authorization prior to the initiation of any trial procedures, after the nature of the trial has been explained according to local regulatory requirements.
- Individuals who can comply with trial procedures and are available for the duration of follow-up.
- All female participants of childbearing potential must have a negative serum beta human chorionic gonadotropin (β-hCG) pregnancy test at screening and a negative urine β-hCG pregnancy test prior to receiving any dose of investigational vaccine/placebo.

Criteria for Exclusion:

Any subject who meets any of the following criteria will not qualify for entry into the trial:

- Subjects and subjects' partners with confirmed ZIKV infection by self-report.
- 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).

  *CDC website defines the information about the flavivirus endemic countries and US regions and territories (see Appendix D for the respective website addresses).
- Known hypersensitivity or allergy to any of the vaccine candidate components (including excipients of the investigational vaccine or placebo).
- Individuals with behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.
- Individuals with any history of progressive or severe neurologic disorder, seizure disorder or neuro-inflammatory disease (eg, Guillain-Barré syndrome).
- Individuals with history or any illness that, in the opinion of the investigator, might interfere with the results of the trial or pose additional risk to the subjects due to participation in the trial.
- Known or suspected impairment/alteration of immune function, including:

  - Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks / ≥2 mg/kg body weight / day prednisone ≥2 weeks) within 60 days prior to Day 1 (use of inhaled, intranasal, or topical corticosteroids is allowed).
  - Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks / ≥2 mg/kg body weight / day prednisone ≥2 weeks) within 60 days prior to Day 1.
  - Receipt of immunostimulants within 60 days prior to Day 1.
  - Receipt of parenteral, epidural or intra-articular immunoglobulin preparation, blood products, and/or plasma derived products within 3 months prior to Day 1 or planned during the full length of the trial. In addition, subjects must be advised not to donate blood during the study period.
  - Known Human Immunodeficiency Virus (HIV) infection or HIV-related disease.
  - Genetic immunodeficiency.
- Individuals with known current or chronic hepatitis B and/or hepatitis C infections.
- Abnormalities of splenic or thymic function.
- Individuals with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
- Individuals with any serious chronic or progressive disease according to judgment of the investigator (eg, neoplasm, insulin-dependent diabetes, cardiac, renal, hepatic or thyroid disease, uncontrolled hypertension, uncontrolled asthma).
- Individuals with body mass index (BMI) greater than or equal to 35 kg/m2 (= weight in kg / [height in meters x height in meters]).
- Individuals participating in any clinical trial with another investigational product, including ZIKV vaccine clinical trial within 30 days prior to first trial visit or intent to participate in another clinical trial at any time during the conduct of this trial.
- Individuals who received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 in this trial or who are planning to receive any vaccine within 28 days of investigational vaccine/placebo administration.
- Individuals involved in the trial conduct or their first degree relatives.
- Individuals with history of substance or alcohol abuse within the past 2 years.
- Female subjects who are pregnant or breastfeeding, or are planning to become pregnant.
- Any positive or indeterminate pregnancy test.
- If female subject of childbearing potential, sexually active, and who has not used any of the "acceptable contraceptive methods" for at least 2 months prior to trial entry:
  - "Of childbearing potential" is defined as status post onset of menarche and not meeting any of the following conditions: menopausal for at least 2 years without any other alternative medical cause (as confirmed by a healthcare professional), status after bilateral tubal ligation for at least 1 year, status after bilateral oophorectomy, or status after hysterectomy.
  - Acceptable birth control methods are defined as one or more of the following:
    - Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring).
    - Barrier (condom with spermicide or diaphragm with spermicide) each and every time during intercourse.
    - Intrauterine device.
    - Monogamous relationship with vasectomized partner. Partner must have been vasectomized for at least six months prior to the subjects' trial entry.
- If female subject of childbearing potential and sexually active, refusal to use an "acceptable contraceptive method" from trial entry through 2 months after the last dose of investigational vaccine/placebo. In addition, female subjects of childbearing potential must be advised not to donate ova during this period.

- To avoid sexual transmission of ZIKV from natural exposure: Refusal to use latex condoms correctly and consistently by sexually active subjects even if other contraceptive measures are used from signing the ICF through the end of the trial. Male subjects must be advised not to donate sperm during this period.

Trial Vaccines:

Investigational Vaccine:

Takeda's PIZV is an aluminum hydroxide-adjuvanted vaccine candidate made from purified formalin-inactivated ZIKV. The virus seed was derived from ZIKV strain PRVABC59 propagated through infection of Vero cells (derived from the kidney of African green monkey) grown in culture, and further purified prior to inactivation. The inactivated purified virus is mixed with aluminum hydroxide to formulate the drug product, PIZV, a liquid formulation filled into single-use vials and stored at 2-8°C. The investigational vaccine is administered IM as a 2-dose regimen of 0.5 mL at 2, 5, or 10 µg antigen per dose, 28 days apart. Refer to the IB for more details.

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 2-8°C. The placebo is administered IM as a 2-dose regimen of 0.5 mL per dose, 28 days apart.

Duration of the Trial:	**Period of Evaluation:**
Approximately 7 or 25 months, following a screening period	7 or 25 months

Main Criteria for Evaluation and Analyses:

The primary endpoints for this trial are

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 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 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.

The secondary endpoints for this trial are

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, and 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, and 6, 12, and 24 months post dose 2) in applicable groups.
- Seroconversion rates (SCR) at 28 days post dose 1 and 28 days post dose 2.

Statistical Considerations:

Analysis sets:

- *Safety Set*: The Safety Set will consist of all randomized subjects who received at least one dose of the investigational vaccine/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 include all subjects in the FAS who have no major protocol violations. The major protocol violation criteria will be defined as part of the blinded data review prior to the unblinding of subject's investigational vaccine/placebo assignment. The categories of major protocol violations include:
    1 not meeting selected entry criteria,
    2 receiving a wrong investigational vaccine/placebo,
    3 receiving prohibited therapies, and
    4 other major protocol violations that may be identified during blinded data reviews.

All summaries and analyses of safety data will be based on subjects in the Safety Set.

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

Analysis of demographics and other baseline characteristics: Summaries of age, gender, race, and other baseline characteristics will be presented by formulation arm.

Immunogenicity analysis: Descriptive statistics for the primary and secondary immunogenicity endpoints, including estimates and 95% confidence intervals (95% CI) for GMT, SPR and SCR, will be provided by time point (28 days post dose 1, 28 days post dose 2, and 6, 12, and 24 months post dose 2 in applicable groups) and by formulation arm. Point estimates and 95% CI for ratios in GMT and differences in SPR and SCR will be provided for each pair of active study arms to aid a single vaccine dose level selection. Immunogenicity summaries and analyses will be provided by dose group overall, as well as by baseline flavivirus serostatus.

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

Safety Analysis: Reactogenicity will be assessed for 7 days following each dose (including day of vaccine/placebo administration) via daily collection of solicited AEs, including local reactions (injection site: pain, erythema, swelling, and induration) and systemic AEs of headache, fatigue, malaise, arthralgia and myalgia. In addition, body temperature (preferably measured as oral temperature) as indicator of reactogenicity will be collected (with fever defined as body temperature ≥38.0°C/100.4°F).

For each solicited AE, including fever, the percentage of subjects will be summarized by event severity for each day for the 7 days after each vaccine/placebo administration and overall. For subjects with more than 1 episode of the same event, the maximum severity will be used for tabulations.

Unsolicited AEs and SAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class (SOC) and preferred term (PT) for each formulation arm including neurological and neuroinflammatory disorders and new medical conditions with onset after the first vaccination.

Safety summaries will be provided by dose group overall, as well as by baseline flavivirus serostatus.

Sample Size Justification:

The sample size was not determined based on formal statistical power calculations.

Interim Analyses:

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, including safety data from all flavivirus primed subjects up to Visit 4 (on Day 29, 28 days post dose 1).

Final Analysis:

The final 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 subsequent analyses will include (1) the data up to Visit 9 (12 months post dose 2) and (2) the data up to Visit 11 (24 months post dose 2). 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). More details regarding the analyses will be provided in the Statistical Analysis Plan (SAP).

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, ie, Visit 11 (Day 757, 24 months post dose 2).

External Data Monitoring Committee:

An external DMC will have oversight of this trial. The external DMC functions at a program level.

The external DMC will consist of 5 independent members and an independent non-voting statistician. External DMC meetings will consist of open and closed face-to-face meetings or teleconference calls. The type and frequency of scheduled meetings will depend on the subject enrollment and safety event rates. Unscheduled ad hoc meetings will occur if a stopping rule occurs, or at any time consultation is requested by the Pharmacovigilance Study Team.

The external DMC will review the safety and tolerability data of all subjects in the flavivirus naïve cohort who have completed Visit 4 (28 days post dose 1) for the purpose of recommendation to start vaccinating flavivirus primed subjects. The external DMC will review safety data on an ongoing basis and when the following set of AEs are reported, the external DMC will review all relevant safety data within 48 hours and consider a possible pause of the study, if it deems necessary:

- Two or more subjects develop the same grade 3 solicited AE starting within 7 days of vaccination
- Two or more subjects develop the same grade 3 unsolicited AE within 28 days of vaccination
- Any SAE
- Any unexpected, serious, or unanticipated risk to the subjects enrolled.

Further information is available in the external DMC charter.

2.1 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 + 3½ 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) indicates 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. Refer to Section 9.1.2.

(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 (see Section 10.0) must be transcribed into the electronic Case Report Form (eCRF). For any procedures at the site, the investigator shall follow his/her standard practice. Refer to Section 9.1.4.

(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. Refer to Section 9.1.5.

(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. Refer to Section 9.1.6.

(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. Refer to Section 9.1.9.

(m) If eligible, the subject will be randomized at Visit 2. Refer to Sections 9.1.3 and 9.1.10.

(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. Refer to Section 9.3.4.

(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. Refer to Section 9.1.7.

(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 (ie, 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.

3.0 TRIAL REFERENCE INFORMATION

3.1 Trial-Related Responsibilities

The sponsor will perform all trial-related activities with the exception of those identified in the Trial-Related Responsibilities template. The identified vendors in the template for specific trial-related activities will perform these activities in full or in partnership with the sponsor.

3.2 Principal Investigator/Coordinating Investigator

The sponsor will select a Signatory Principal Investigator / Coordinating Investigator from the investigators who participate in the trial. Selection criteria for this investigator will include significant knowledge of the trial protocol, the investigational vaccine, their expertise in the therapeutic area and the conduct of clinical research, as well as trial participation. The Signatory Principal Investigator / Coordinating Investigator will be required to review and sign the clinical study report and by doing so agrees that it accurately describes the results of the trial.

3.3 List of Abbreviations

ADE	Antibody-Dependent Enhancement
AE	Adverse Event
BARDA	Biomedical Advanced Research and Development Authority
BMI	Body Mass Index
CI	Confidence Interval
CNS	Central Nervous System
CRO	Contract Research Organization
CZS	Congenital Zika Syndrome
DENV	Dengue Virus
DMC	Data Monitoring Committee
eCRF	electronic Case Report Form
EDC	Electronic Data Capture system
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full Analysis Set
FDA	U.S. Food and Drug Administration
FRNT	Focus Reduction Neutralizing Test
GBS	Guillain-Barré syndrome
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
β-hCG	beta human Chorionic Gonadotropin
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ID	Identification
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IM	Intramuscular
IRB	Institutional Review Board
IRT	Interactive Response Technology
JE	Japanese Encephalitis
LOD	Limit of Detection
MAC-ELISA	IgM-Antibody Capture Enzyme-Linked Immunosorbent assay

MedDRA	Medical Dictionary for Regulatory Activities
MN	Micro-neutralization
NHP	Non-Human Primate
PBS	Phosphate Buffered Saline solution, ie, water-based salt solution containing potassium dihydrogen phosphate (KH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), and sodium chloride
PHEIC	Public Health Emergency of International Concern
PIZV	Purified Inactivated Zika Virus Vaccine candidate
PPS	Per-Protocol Analysis Set
PRNT	Plaque Reduction Neutralization Test
PT	Preferred Term
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
RVP	Reporter Virus Particle
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SOC	System Organ Class
SPR	Seropositivity Rate
SUSAR	Suspected Unexpected Serious Adverse Reaction
WHO	World Health Organization
WNV	West Nile Virus
WRAIR	Walter Reed Army Institute of Research
YF	Yellow Fever
ZIKV	Zika Virus

3.4 Corporate Identification

Not applicable.

4.0 INTRODUCTION

4.1 Background

4.1.1 Zika Virus

Zika virus (ZIKV) is a mosquito-borne single-stranded positive-sense ribonucleic acid (RNA) virus (11 kb in size) (26) which belongs to the *Flaviviridae* family, genus *Flavivirus*, that often causes no or only mild symptoms, including a rash and a febrile flu-like illness in the majority of symptomatic individuals. The ZIKV virion shows icosahedral symmetry of its nucleocapsid (27) and presents structural similarities with other members of the *Flaviviridae*, including Spondweni virus, Yellow Fever (YF) virus, Dengue virus (DENV), West Nile virus (WNV), Japanese Encephalitis (JE) virus, and Tick-borne Encephalitis virus. The genome is translated into a single polyprotein subsequently cleaved by both viral and host cell enzymes into three structural (capsid, pre-membrane/membrane and envelope) proteins that form the virion and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) (28).

ZIKV was discovered in 1947 in Uganda while testing samples taken from sentinel rhesus monkeys used as bait during a surveillance of YF in the Zika Forest near Entebbe in Uganda. The first human disease cases were reported in 1952 in Uganda (29) and Tanzania (30). For as long as 55 years, the virus circulated in areas of Africa, Southeast Asia, and the Pacific Islands, and was considered a rare disease with mild symptoms (31); only 14 human cases were reported and no outbreaks were detected during that period (32).

4.1.2 Epidemiology

The first large outbreak of ZIKV disease was reported in Yap Island (Federal state of Micronesia) in 2007 (33), and was followed by an outbreak in French Polynesia in 2013-2014 (34). The first cases of ZIKV disease in South America were reported in Easter Island in 2014 (35), and the first reports of locally transmitted infection (ie, by mosquito) came from Brazil in May 2015 (36), which triggered the third outbreak in 2015-2016. Since then, ZIKV disease has continued spreading through the South American continent and to other regions. In the current outbreak that started in 2015, there have been estimates of more than 750,000 ZIKV-suspected cases reported from more than 60 countries/territories; most of these cases are from the South America continent (37, 38).

4.1.3 Transmission

The primary mode of transmission of ZIKV is through the bite of female mosquitoes, prominently *Aedes aegypti* and *Ae. albopictus* species that have spread globally (39). Other potential vectors for ZIKV include other *Aedes* species (including *Ae. furcifer*, *Ae. vittatus*, *Ae. dalzieli*, *Ae. metallicus*, *Ae. hirsutus*, *Ae. unilinaetus*, *Ae. africanus*, *Ae. taylori*, *Ae. hensilli*, and *Ae. luteocephalus*) along with mosquitoes from other genera (such as *Anopheles*, *Mansonia*, and *Culex*) (40), the true extent of the vectors being still unknown. Nonhuman and human primates are likely the main reservoirs of the ZIKV as for most arthropod-borne viruses (arboviruses) (41), and

anthroponotic (human-to-vector-to-human) transmission is most likely responsible for the rapid spreading of the virus during outbreaks.

Vertical transmission from mother to fetus during pregnancy (6, 42-44) and the perinatal period (45, 46), sexual contact (47-49), and blood transfusion (50, 51) are also confirmed modes of transmission of the disease. Potentially, the virus could be transmitted through laboratory exposure and by body fluid contact (52, 53), eg, tears, urine (54-56), breast milk, or through solid organ transplants (57, 58), but there is limited evidence to support this (59-61).

4.1.4 ZIKV Infection: Detection, Disease, Complications and Treatment

Detection

Testing for ZIKV infection is complicated by the temporal appearance and disappearance of biologic analytes in the infected person and thus multiple tests and sample types are often needed to establish a definitive laboratory diagnosis of ZIKV infection. Viral RNA is the first analyte that can be detected in an infected person in multiple specimen types. In blood, as the immune response develops (immunoglobulin M [IgM] titers rise), levels of viral RNA decline. However, viral RNA may be detectable in some infected persons for longer periods in certain specimen types (62, 63).

Whole blood, serum and urine are the primary diagnostic specimens for ZIKV infection. Other specimen types such as plasma, cerebrospinal fluid, and amniotic fluid are authorized for use with some tests that have received a Food and Drug Administration (FDA) Emergency Use Authorization (64). Other specimens that still need validation but might be appropriate for evaluating ZIKV disease include saliva (65, 66), semen, peripheral blood mononuclear cells, amniotic fluid (43), and tissue samples of, for instance, fetal brain and placenta biopsies (6, 42, 67).

Testing methods used for ZIKV infection include molecular and serologic testing: reverse transcription-polymerase chain reaction (RT-PCR) for viral nucleic acid, and IgM-antibody capture enzyme-linked immunosorbent assay (MAC-ELISA), as well as focus reduction neutralizing test (FRNT), plaque reduction neutralization test (PRNT), micro-neutralization (MN) assay, and reporter virus particle (RVP) assay for anti-ZIKV antibodies. Although PRNT typically provides good specificity, serological assays are subject to cross-reactivity, especially in subjects with prior flavivirus infection or immunization history. In contrast, diagnosis by RT-PCR has been most successful within 1 week after the onset of clinical illness, though viremia is generally low level, which makes viral isolation from clinical samples difficult (68). Data from studies in non-human primates (NHP) evaluated for neutralizing antibodies by conventional FRNT, MN assay and RVP assay suggest that although both FRNT and MN assay strongly correlated with EC_{50} RVP values, RVP assay was more sensitive (69).

Disease

More than 80% of ZIKV infections remain asymptomatic (33), and for the symptomatic cases, the disease is in general mild and of short duration. Some clinical manifestations include, but are not limited to, mild fever, maculopapular rash, conjunctivitis and arthralgia (70).

The incubation period between infection and symptoms appears to be of 3 to 11 days (3, 4), which is similar to that of other mosquito-borne flavivirus diseases. The course of infection is mostly self-limiting. However, pre-existing cross-reactive antibodies directed against other flaviviruses may exacerbate secondary ZIKV infection through antibody-dependent enhancement (ADE). A recent publication has demonstrated that human dengue virus antibodies enhance in vitro infection with ZIKV, suggesting that ADE is not merely a theoretical concern (71).

Congenital Zika Syndrome

Despite mild clinical symptoms in the pregnant woman, ZIKV infection during pregnancy has been associated with serious outcomes for the fetus and newborn. The severity of the disease is related to the consequences in the fetus and newborn child from women with ZIKV infection during pregnancy (42, 72). The spectrum of congenital anomalies associated with ZIKV infection, known as Congenital Zika Syndrome (CZS), consists of severe microcephaly (5-8) with partially collapsed skull, cerebral cortices with subcortical calcifications, macular scarring and focal pigmentary retinal mottling, congenital contractures, and marked early hypertonia with symptoms of extrapyramidal involvement (9).

The exposure to ZIKV at any moment during pregnancy is a risk for fetal or postnatal developmental complications, primarily affecting brain development. Recent analysis of the U.S. Zika Pregnancy Registry (USZPR) showed that the prevalence of newborns with congenital defects from pregnancies with laboratory-confirmed ZIKV infection is 10% (95% CI: 7%-14%). This is 30 times higher than baseline prevalence before more recent Zika outbreaks (10).

Since the beginning of these outbreaks and up to end of September 2017, there have been 3689 confirmed CZS malformations potentially associated with ZIKV infection in 27 countries and territories of Latin America and the Caribbean.

Guillain-Barré Syndrome

ZIKV is a neurotropic flavivirus that can potentially cause disease within the central nervous system. Worldwide concern over ZIKV causing Guillain-Barré Syndrome (GBS) emerged when an increased number of GBS cases was temporally associated with the 2013-2014 ZIKV outbreak in French Polynesia, during which 42 GBS cases were reported, all of these cases had neutralizing antibodies against ZIKV, compared to 56% in the control group ($p<0.0001$) (11-13). Other neurological complications, such as encephalitis, meningoencephalitis (14), paresthesia, facial paralysis and myelitis (15, 16), have also been associated with ZIKV infection. Based on these findings, the countries affected by ZIKV disease in the 2015-2016 outbreak improved and extended the surveillance of neurological syndromes. In 2017, after the spread of ZIKV throughout the Pacific Islands, Latin America and the Caribbean, a total of 200 cases of GBS related to ZIKV infection have been reported in these regions as case series or isolated cases. The incidences reported have increased 2 to 9.8 times compared to baseline before the epidemic in Latin America (19), and up to 20 times in French Polynesia (12). The association of GBS was only reported when the circulating strain was of Asian lineage (21). As of 01 February 2017, ZIKV of the Asian lineage was circulating in more than 80% of affected countries, including 48 countries in

Latin America (22). The overall incidence of ZIKV-associated GBS is estimated to be 24 per 100,000 cases of ZIKV infection (23).

Treatment

No specific antiviral treatment is available for ZIKV infections and no vaccine against ZIKV is currently available (73). As the disease is self-limiting, treatment for uncomplicated ZIKV infection is supportive and focuses on symptoms. Treatment for more severe forms of ZIKV infection should address specific medical issues, and in the case of congenital ZIKV infection, neurodevelopmental issues for the infant's particular needs (74).

Patient care includes proper hydration, monitoring for possible coagulopathy and multiple organ failure. Intensive care is recommended for the patients depicting serious conditions, such as showing signs of coagulopathy, tachycardia, hypotension and renal dysfunction. Patients with neurological complications such as GBS require hospitalization (75). Non-steroidal anti-inflammatory drugs and aspirin can be recommended for the treatment. Such medications should be avoided in case of concomitant infection with dengue fever virus. In case of suspected ZIKV infection in pregnant women, sonography is recommended to monitor the proper fetal growth and potential complications of infant microcephaly (76).

The main recommendations to address outbreaks are through prevention (avoiding mosquito bites, reducing sexual transmission, improving the procedures for safe blood transfusion), and vector control measures (77, 78).

4.2 Rationale for the Proposed Trial

4.2.1 Medical Need

ZIKV has posed a challenging situation for health, public and economic sectors of affected countries. The increase in microcephaly cases and other neurological disorders reported in Brazil following a similar cluster in the French Polynesia in 2014 (24), prompted the World Health Organization (WHO) to declare Zika a Public Health Emergency of International Concern (PHEIC) on 01 February 2016 (25) and recommended to focus research on the causal association of ZIKV infection during pregnancy and microcephaly and other neurological abnormalities in newborns. WHO recommended to standardize and enhance the surveillance for microcephaly and GBS particularly in areas of known or high risk of ZIKV transmission and to intensify the research into the etiology of new clusters of microcephaly and other neurological disorders to determine whether there is a causative link to ZIKV and/or other factors or co-factors.

Considering the rapid spread of ZIKV and the significant threat to public health especially to pregnant women and their children's health, the U.S. Department of Health and Human Services declared Zika a public health emergency in Puerto Rico in August 2016 (79). In November 2016, after evaluating the evidence about the causal association of ZIKV infection during pregnancy and microcephaly and other neurological abnormalities in newborns, WHO announced that Zika no longer represents a PHEIC (80) but stressed the need to escalate the disease to a sustained work program with dedicated resources to address its long-term nature and its associated consequences.

The risk of infection with ZIKV is increasing given that the spread of ZIKV is rapid and intense in tropical and subtropical countries. Vector control is difficult to establish and maintain, and new areas continue to be colonized by the *Aedes* species. Climate changes may also create favorable environments to maintain the vector (81). There is an increased urban population and mobility, and the role of sexual transmission may be an important mechanism to spread rapidly the virus into susceptible populations, even in regions where the vector is uncommon.

Considering the conclusive associations between ZIKV infections and fetal and postnatal abnormalities, as well as strong associations with other medically important complications such as GBS, the development of a vaccine that can provide protection is crucial for countries where the epidemic is expected to arrive and/or persist, as well as in countries in which the virus has not yet been introduced. In order to address the urgent medical need and in anticipation of possible additional outbreaks with rapid onsets, Takeda has initiated the development of a purified inactivated ZIKV vaccine candidate, PIZV, for use in endemic areas and non-endemic areas for prevention of ZIKV associated illness of any severity and/or infection.

4.2.2 BARDA Funding Award

The Biomedical Advanced Research and Development Authority (BARDA) from the U.S. Department of Health and Human Services' Office of the Assistant Secretary for Preparedness and Response is providing funding to Takeda for the US-based PIZV development program (82).

4.2.3 Vaccines Under Development

Currently, there is no approved vaccine against ZIKV. Various companies and academic organizations are using a variety of vaccine approaches, including inactivated virus, virus-like particles, nucleic-acid-based vaccines, live vectored vaccines, subunit vaccines, and live recombinant approaches. Most of these are in preclinical development and many are expected to enter Phase I clinical studies in 2017.

4.2.4 Summary of Available Non-clinical Data

There are no non-clinical data currently available for PIZV.

PIZV will be tested for nonclinical safety in a 3-dose Good Laboratory Practice-compliant, repeat-dose toxicity and local tolerance study in New Zealand White Rabbits. Toxicology data will be available prior to study start and provided in the Investigator's Brochure (IB).

4.2.5 Summary of Available Clinical Data

There are no clinical data currently available for PIZV.

4.2.6 Takeda Vaccine Candidate

Takeda's PIZV is an aluminum hydroxide (alum)-adjuvanted vaccine candidate made from purified formalin-inactivated ZIKV. The virus seed was derived from ZIKV strain PRVABC59 propagated through infection of Vero cells (derived from the kidney of African green monkey) grown in culture, and further purified prior to inactivation. The choice of ZIKV strain may not be a

critical parameter as evidence suggests that infection with a single ZIKV strain can elicit broadly neutralizing antibodies across different strains (83).

Refer to Section 8.1 for additional details.

4.2.7 Rationale for ZIK-101 Trial

The proposed ZIK-101 clinical trial is an observer-blind placebo-controlled first-in-human study.

The rationale for the observer-blind approach is based on the different physical appearance of the investigational vaccine compared to the saline solution placebo that was selected and preferred to alum-containing placebo to avoid side effects associated with alum. The rationale for the use of placebo in this trial is based on the absence of effective treatment or prevention for ZIKV disease.

Due to the need to vaccinate women and men of reproductive age, the primary target population of PIZV will be 9 years of age and older. In this phase 1 study, study population is limited to ≥18 to ≤49 years, and age expansion will be included in the next phase of development.

To ensure the enrollment of healthy adults, screening tests (hematology, biochemistry and urinalysis) will be performed prior to vaccine administration.

Flavivirus primed adults are included in this study to assess if there is any impact from pre-existing flavivirus antibodies. The testing of flaviviral serological status will be performed prior to randomization and will enable stratification of randomization.

Due to the ongoing threat of ZIKV disease and the urgent need of a vaccine, ZIK-101 will be accelerated to assess dose finding for PIZV, in addition to evaluation of safety and immunogenicity of the vaccine candidate (PIZV) in both flavivirus seronegative (naïve) and seropositive (primed) subjects.

The reactogenicity/safety of PIZV is unknown. Flavivirus naïve subjects will be enrolled during the first phase of the study. In the absence of clinical data on ZIKV vaccine, it is proposed to recruit flavivirus primed subjects in a second stage. If positive recommendations from an external Data Monitoring Committee (DMC) are issued following the review of safety and tolerability data in the flavivirus naïve population, when the last flavivirus naïve subject has completed study Visit 4 (28 days post dose 1), vaccination of flavivirus primed subjects will start.

The primary rationale for selection of the three vaccine dose levels can be summarized as follows:

- the low-dose level (2 µg) was selected for purposes of potentially identify an immunological threshold, and based on the limit of the analytical methods for the drug product available at the time;
- the mid-dose level (5 µg) was selected to provide a mid-point on the dose response curve. In addition, this dose level is based on the Walter Reed Army Institute of Research (WRAIR) vaccine currently tested in Phase 1 (84), which was originally based on initial studies in NHPs (85); and
- the high-dose level (10 µg) was selected to follow an approximate 2-fold range and to further define the potential dose-response curve in both naïve and primed subjects.

The initial immunogenicity and efficacy studies in mice support the rationale for formulating PIZV with aluminum hydroxide adjuvant in order to elicit a robust immune response and survival following wild-type challenge.

PIZV dose will be selected based on descriptive safety data and geometric mean titers (GMT) of neutralizing anti-ZIKV antibody levels at 28 days post dose 2 in both flavivirus naïve and primed subjects.

The trial will be conducted in accordance with the protocol, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) GCP Guidelines and applicable regulatory requirements.

5.0 TRIAL OBJECTIVES AND ENDPOINTS

5.1 Objectives

5.1.1 Primary Objectives

- To describe the safety of two doses of 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

5.1.2 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

5.2 Endpoints

5.2.1 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 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.

Immunogenicity of PIZV, as determined by:

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

5.2.2 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, and 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, and 6, 12, and 24 months post dose 2) in applicable groups.
- Seroconversion rates (SCR) at 28 days post dose 1 and 28 days post dose 2.

6.0 TRIAL DESIGN AND DESCRIPTION

6.1 Trial 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, in ZIKV endemic and non-endemic regions.

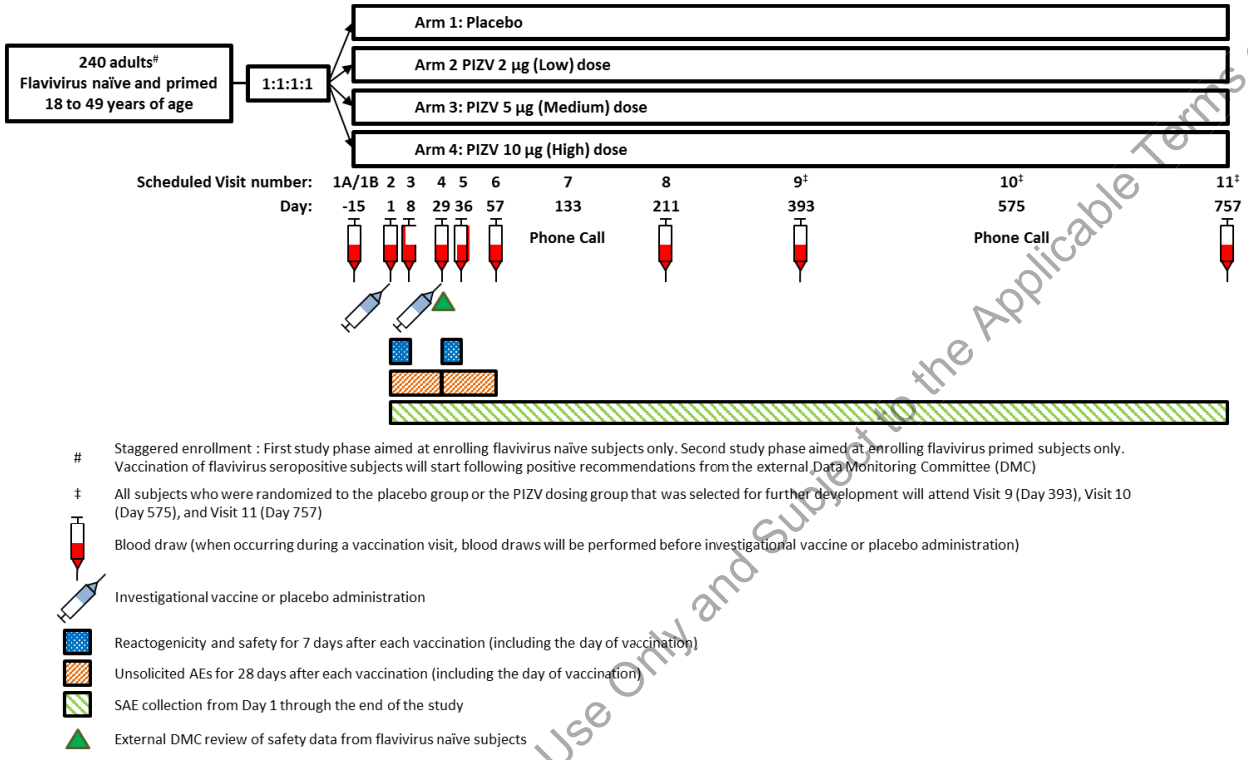
The trial will last approximately 7 or 25 months for each subject, following a screening period prior to Visit 2. The screening period will last for up to 2 weeks (14 days) prior to randomization, with the possibility that some subjects (Flavivirus primed subjects only) will need to repeat some screening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

- Flavivirus naïve subjects: subjects without detectable serum antibodies against a panel of flaviviruses as measured by a reactive antibody-based assay (Luminex) (refer to the Serology Plan, available on file).
- Flavivirus primed subjects: subjects with serum antibodies against a panel of flaviviruses as measured by a reactive antibody-based assay (Luminex) (refer to the Serology Plan, available on file).

Approximately 240 subjects aged ≥18 to ≤49 years 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 versus ≥30 to ≤49 years.

A schematic representation of the trial design is included as Figure 6.a. A schedule of trial procedures is provided in Section 2.1.

Figure 6.a Schematic Representation of Trial Design

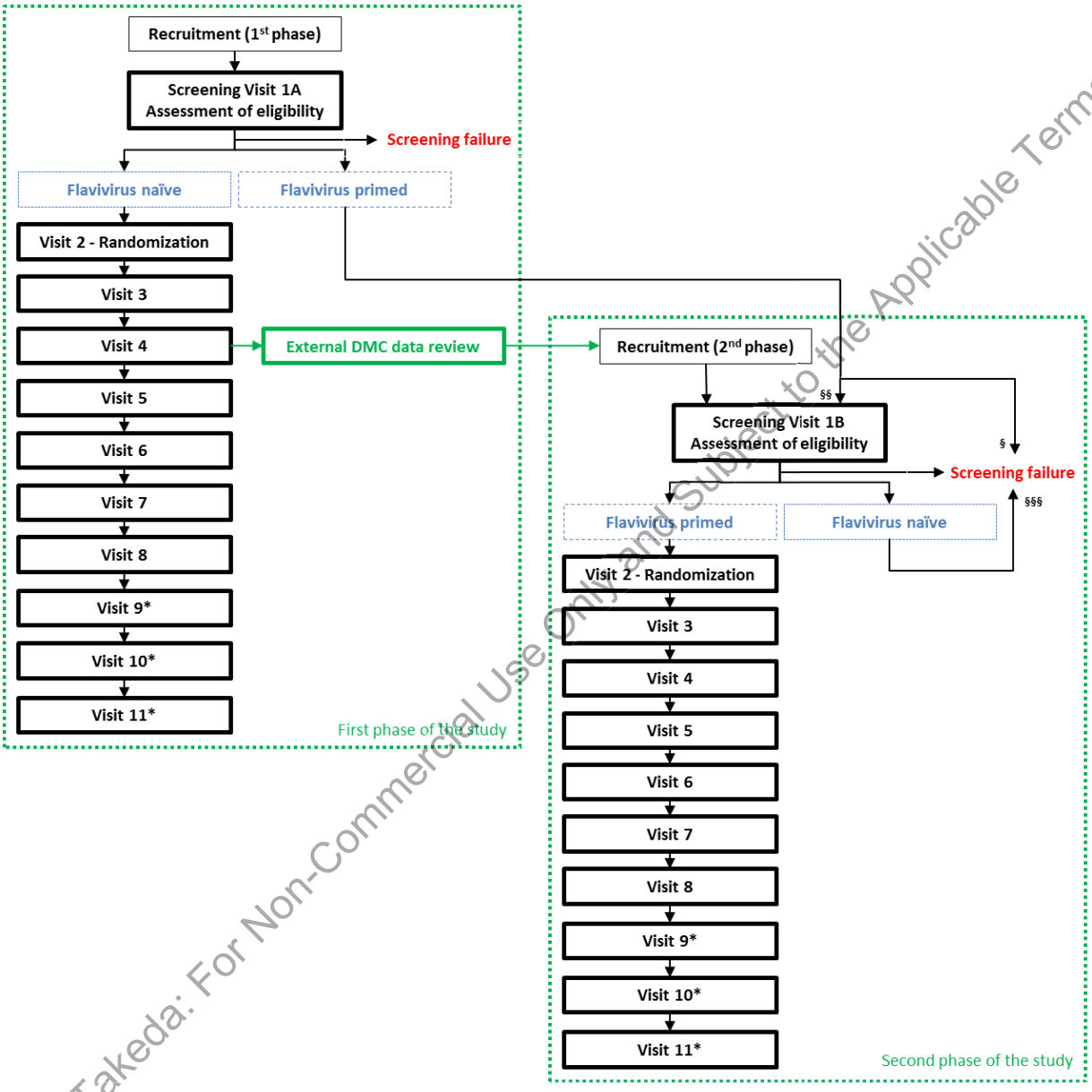


Staggered enrollment (Figure 6.b): There will be two study phases.

- 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 (ie, 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 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. Therefore, it is recommended that the key aspects of the ICF already signed during the Screening Visit 1A are reinforced with the subject. 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 6.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 6.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 6.b §§§).

Figure 6.b Staggered Enrollment



* 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
- 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: ie, 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. Refer to Section 9.1.6 and the Serology Plan (available on file).

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 at Visits 2 and 4 before each investigational vaccine/placebo administration for pregnancy testing. Refer to Section 9.1.6.

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.

Refer to Section 13.2 for details about sequence of analyses.

6.2 Justification for Trial Design, Dose, and Endpoints

The trial design and the collection of solicited and unsolicited AEs following vaccination are consistent with guidelines on vaccine evaluation trials, including dose-finding studies.

The design of the study answers to the principles outlined in the ICH Considerations on Clinical Trials E8 (86) and the WHO Guidelines for Evaluation of Vaccines (87), and more specifically for phase 1 trials. As such, the study design, objectives and endpoints were defined so as to assess the initial safety and reactogenicity of the vaccine candidate, obtain preliminary information on its immunogenicity, and provide data useful for the design of further clinical studies. The dose-ranging design adopted is in line with the ICH and FDA guidance (88, 89). The use of placebo as control for the investigational vaccine is justified in the absence of a proven intervention (90), ie, as no prophylactic vaccine against ZIKV infection is available to date.

As this is a first in human trial, a relatively high serum volume (60 mL) will be needed for assay development and other pre-clinical studies to support possible alternative license pathways which may include bridging animal data to human data.

The rationale for the proposed trial is given in Section 4.2.7.

Please also refer to the IB.

6.3 Duration of Subject's Expected Participation in the Entire Trial

Each enrolled subject's participation in the trial will last approximately 7 or 25 months, following a screening period prior to Visit 2 of two-weeks for flavivirus naïve subjects and of more than two weeks for flavivirus primed subjects.

6.4 Premature Termination or Suspension of Trial or Investigational Site

6.4.1 Criteria for Premature Termination or Suspension of the Trial

The trial will be completed as planned unless one or more of the following criteria are satisfied that require temporary suspension or early termination of the trial.

- New information or other evaluation regarding the safety or efficacy of the investigational vaccine that indicates a change in the known risk/benefit profile, such that the risk/benefit is no longer acceptable for subjects participating in the trial.
- The external DMC recommends that the trial should be suspended or terminated.
- Significant deviation from Good Clinical Practice (GCP) that compromises the ability to achieve the primary trial objectives or compromises subject safety.

6.4.2 Criteria for Premature Termination or Suspension of Investigational Sites

A trial site may be terminated prematurely or suspended if the site (including the investigator) is found in significant deviation from GCP, protocol, or contractual agreement, is unable to ensure adequate performance of the trial, or as otherwise permitted by the contractual agreement.

6.4.3 Procedures for Premature Termination or Suspension of the Trial or the Participation of Investigational Site(s)

In the event that the sponsor, an institutional review board (IRB)/independent ethics committee (IEC) or regulatory authority elects to terminate or suspend the trial or the participation of an investigational site, a trial-specific procedure for early termination or suspension will be provided by the sponsor; the procedure will be followed by applicable investigational sites during the course of termination or trial suspension.

7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF SUBJECTS

All entry criteria, including test results, need to be confirmed prior to randomization.

7.1 Inclusion Criteria

Subject eligibility is determined according to the following criteria:

1. The subject is aged ≥18 to ≤49 years.
2. Individuals who are in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs) and eligibility screening tests (hematology, biochemistry and urinalysis) and clinical judgment of the investigator. Vital signs must be within normal limits (ie, below Grade 1 as specified in the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers, see Appendix E). Screening tests must be within normal limits or not be above Grade 1 as defined in the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers (see Appendix E).
3. The subject signs and dates a written, informed consent form and any required privacy authorization prior to the initiation of any trial procedures, after the nature of the trial has been explained according to local regulatory requirements (Appendix B).
4. Individuals who can comply with trial procedures and are available for the duration of follow-up.
5. All female participants of childbearing potential must have a negative serum beta human chorionic gonadotropin (β-hCG) pregnancy test at screening and a negative urine β-hCG pregnancy test prior to receiving any dose of investigational vaccine/placebo.

7.2 Exclusion Criteria

Any subject who meets any of the following criteria will not qualify for entry into the trial:

1. Subjects and subjects' partners with confirmed ZIKV infection by self-report.
2. 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).

   *CDC website defines the information about the flavivirus endemic countries and US regions and territories (see Appendix D for the respective website addresses).
3. Known hypersensitivity or allergy to any of the vaccine candidate components (including excipients of the investigational vaccines, control or placebo as summarized in Section 8.1).
4. Individuals with behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.
5. Individuals with any history of progressive or severe neurologic disorder, seizure disorder or neuro-inflammatory disease (eg, Guillain-Barré syndrome).

6. Individuals with history or any illness that, in the opinion of the investigator, might interfere with the results of the trial or pose additional risk to the subjects due to participation in the trial.
7. Known or suspected impairment/alteration of immune function, including:
    - Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks / ≥2 mg/kg body weight / day prednisone ≥2 weeks) within 60 days prior to Day 1 (use of inhaled, intranasal, or topical corticosteroids is allowed).
    - Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks / ≥2 mg/kg body weight / day prednisone ≥2 weeks) within 60 days prior to Day 1.
    - Receipt of immunostimulants within 60 days prior to Day 1.
    - Receipt of parenteral, epidural or intra-articular immunoglobulin preparation, blood products, and/or plasma derived products within 3 months prior to Day 1 or planned during the full length of the trial. In addition, subjects must be advised not to donate blood during the study period.
    - Known Human Immunodeficiency Virus (HIV) infection or HIV-related disease.
    - Genetic immunodeficiency.
8. Individuals with known current or chronic hepatitis B and/or hepatitis C infections.
9. Abnormalities of splenic or thymic function.
10. Individuals with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
11. Individuals with any serious chronic or progressive disease according to judgment of the investigator (eg, neoplasm, insulin-dependent diabetes, cardiac, renal, hepatic or thyroid disease, uncontrolled hypertension, uncontrolled asthma).
12. Individuals with body mass index (BMI) greater than or equal to 35 kg/m2 (= weight in kg / [height in meters x height in meters]).
13. Individuals participating in any clinical trial with another investigational product, including ZIKV vaccine trial, within 30 days prior to first trial visit or intent to participate in another clinical trial at any time during the conduct of this trial.
14. Individuals who received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 in this trial or who are planning to receive any vaccine within 28 days of investigational vaccine/placebo administration.
15. Individuals involved in the trial conduct or their first degree relatives.
16. Individuals with history of substance or alcohol abuse within the past 2 years.
17. Female subjects who are pregnant or breastfeeding, or are planning to become pregnant.
18. Any positive or indeterminate pregnancy test.

19. If female subject of childbearing potential, sexually active, and who has not used any of the "acceptable contraceptive methods" for at least 2 months prior to trial entry:
    - "Of childbearing potential" is defined as status post onset of menarche and not meeting any of the following conditions: menopausal for at least 2 years without any other alternative medical cause (as confirmed by a healthcare professional), status after bilateral tubal ligation for at least 1 year, status after bilateral oophorectomy, or status after hysterectomy.
    - Acceptable birth control methods are defined as one or more of the following:
    - Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring).
    - Barrier (condom with spermicide or diaphragm with spermicide) each and every time during intercourse.
    - Intrauterine device.
    - Monogamous relationship (only have one sexual partner) with vasectomized partner. Partner must have been vasectomized for at least six months prior to the subjects' trial entry.
20. If female subject of childbearing potential and sexually active, refusal to use an "acceptable contraceptive method" from trial entry through 2 months after the last dose of investigational vaccine/placebo. In addition, female subjects of childbearing potential must be advised not to donate ova during this period.
21. To avoid sexual transmission of ZIKV from natural exposure: Refusal to use latex condoms correctly and consistently by sexually active subjects even if other contraceptive measures are used from signing the ICF through the end of the trial. Male subjects must be advised not to donate sperm during this period.

Temporary screening failure

A subject may be a temporary screen failure under the following circumstances: due to temporary conditions such as receipt of immunosuppressive or immunomodulatory therapies, blood products, immunoglobulins, or vaccines, in the respective prohibited periods (see exclusion criteria 7, 13 or 14), or in the event that a subject has an indeterminate pregnancy test result or has not used acceptable contraceptive methods in the period defined in the protocol, prior to the start of the study (exclusion criteria 19). Subjects screen failed for the above-mentioned reasons may be rescreened once they cease to meet the respective exclusion criteria. Should the respective repeated test results be available within the initial screening period, no new subject ID numbers will be required; the repeated screening will be recorded in the appropriate CRF (unscheduled lab form). Should the respective repeated test results not be available within the initial screening period, the subject would need to be screen failed and all screening procedures and tests would need to be repeated with a new subject ID number.

7.3 Criteria for Delay of Vaccination and/or Blood Sampling

After enrollment, subjects may encounter clinical circumstances that warrant a delay in the administration of the subsequent dose of investigational vaccine/placebo. These situations are listed below:

- Individuals with a 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.
- Individuals who received any other vaccines within 28 days of planned vaccination.

In the event that a subject meets a criterion for delay of vaccination, the subject may receive trial vaccination once the window for delay has passed as long as the subject is otherwise eligible for trial participation. The decision to vaccinate in those situations will be taken by the investigator.

There are also circumstances under which receipt of further vaccines is a contraindication in this trial. These circumstances include anaphylaxis or severe hypersensitivity reactions following the initial vaccine/placebo administration. If these reactions occur, the subject must not receive additional vaccinations; however, the subject is encouraged to continue in trial participation for safety reasons.

7.4 Criteria for Early Study Termination of a Subject

Under some circumstances, a subject's trial participation may be terminated early. This means that no further trial procedures (including data collection) will be performed on that subject beyond the specific date of early termination. The primary reason for early termination of the subject from the trial should be recorded in the electronic Case Report Form (eCRF "end of study visit" page) using the following categories. For screen failure subjects, refer to Section 9.1.10.

1. Adverse Event: The subject has experienced an AE (irrespective of being related / unrelated to the Trial Vaccine or trial-related procedures) that requires early termination because continued participation imposes an unacceptable risk to the subject's health and / or the subject is unwilling to continue participation because of the AE. If the subject is unwilling to continue because of the AE the primary reason for early termination in this case will be 'withdrawal due to AE' and not 'withdrawal of consent', see below. Any ongoing AEs leading to early termination will be followed by the investigator until resolution or stabilization.
2. Lost to follow-up: The subject did not return to the clinic and at least three attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented.
3. Withdrawal of consent: The subject wishes to withdraw from the trial. The primary reason for early termination will be 'withdrawal of consent" if the subject withdraws from participation due to a non-medical reason (ie, reason other than AE). The reason for withdrawal, if provided, should be recorded in the eCRF.

Note: All attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be recorded.

4. Premature study termination by sponsor, a regulatory agency, the IEC/IRB, or any other authority.

If the clinical study is prematurely terminated by the sponsor, the investigator is to promptly inform the study subjects and local EC/IRB and should assure appropriate follow up for the subjects. The primary reason for early termination in this case will be 'trial termination".

5. Subject's death during trial participation.
6. Other.

   Note: The specific reasons should be recorded in the "specify" field of the eCRF.

7.5 Criteria for Premature Discontinuation of Trial Vaccine Application

Early (premature) study termination of a subject will by default prevent the subject from continued Trial Vaccine administration, as the subject will no longer be participating in the study.

In addition to early termination (see Section 7.4) criteria, other situations may apply in which subjects may continue participating in the trial (eg, contributing safety data according to protocol) but Trial Vaccine application is discontinued selectively. Regardless of the reasons for discontinuation of Trial Vaccine application, this must be documented as protocol deviation. Even if the subject is deemed ineligible to continue to receive Trial Vaccine, all efforts should be made to continue the collection of safety data according to protocol. In addition, the one primary reason for premature discontinuation of Trial Vaccine application should be recorded in the eCRF, "end of Trial Vaccine application" page using the following categories.

1. Adverse Event: The subject has experienced an AE (irrespective of being related / unrelated to the Trial Vaccine or trial-related procedures) for which subsequent Trial Vaccine applications impose an unacceptable risk to the subject's health, but the subject will continue trial participation for safety, or a subset of other study procedures.
2. Lost to follow-up: The subject did not return to the clinic and at least three attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented
3. Withdrawal of consent: The subject wishes to withdraw from the trial. The primary reason for early termination will be 'withdrawal of consent" if the subject withdraws from participation due to a non-medical reason (ie, reason other than AE). The reason for withdrawal, if provided, should be recorded in the eCRF.
4. Premature study termination by sponsor, a regulatory agency, the IEC/IRB, or any other authority.

   If the clinical study is prematurely terminated by the sponsor, the investigator is to promptly inform the study subjects and local EC/IRB and should assure appropriate follow up for the subjects. The primary reason for early termination in this case will be 'trial termination".
5. Subject´s death during trial participation.

6. Protocol deviation: A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. The subject may remain in the trial unless continuation in the trial jeopardizes the subject's health, safety or rights (see Section 7.4).
7. Pregnancy: Any subject who, despite the requirement for adequate contraception, becomes pregnant during the trial will not receive further Trial Vaccine applications. Pregnant subjects should, however, be asked to continue participating in the trial contributing data to the safety follow-up according to protocol. In addition, the site should maintain contact with the pregnant subject and complete a "Clinical Trial Pregnancy Form" as soon as possible. The subject should be followed-up until the birth of the child, or spontaneous or voluntary termination; when pregnancy outcome information becomes available, the information should be captured using the same form. Data obtained from the "Clinical Trial Pregnancy Form" will be captured in the safety database.
8. Other.

   Note: The specific reasons should be recorded in the "specify" field of the eCRF.

8.0 CLINICAL TRIAL MATERIAL MANAGEMENT

This section contains information regarding all vaccines and materials provided directly by the sponsor, and/or sourced by other means, that are required by the trial protocol, including important sections describing the management of clinical trial material.

8.1 Trial Vaccines and Materials

8.1.1 Dosage Form, Manufacturing, Packaging, and Labeling

In this protocol, the investigational vaccine (PIZV) refers to Zika purified formalin-inactivated virus formulated with 200 µg aluminum hydroxide, $Al(OH)_3$, as adjuvant, in phosphate buffered saline solution (PBS). The final liquid formulated product is filled into single-use vials and sealed with tamper-evident seals at Takeda's manufacturing plant in Hikari, Japan. The vaccine is packaged, labeled, and shipped to unblinded study site pharmacist/designee. The investigational vaccine is administered IM as a 2-dose regimen of 0.5 mL at 2, 5, or 10 µg antigen per dose, 28 days apart. Refer to the IB and the Pharmacy Manual for more details.

Sodium chloride (NaCl) 0.9% solution for injection is being used as placebo. It is supplied in single-use vials. It is a sterile, clear, colorless liquid solution of sodium chloride without preservative designed for parenteral use only. The placebo is packaged, labelled and shipped to unblinded study site pharmacist/designee. The placebo is administered IM as a 2-dose regimen of 0.5 mL per dose, 28 days apart. Refer to the Pharmacy Manual for more details.

8.1.2 Storage

PIZV/placebo will be shipped in refrigerated and temperature-monitored containers. From receipt and prior to use, the investigational vaccine and the placebo must be protected from light and stored at 2°C to 8°C in a temperature monitored refrigerator with controlled access available only to authorized trial personnel. The investigational vaccine/placebo must not be frozen. In case of accidental freezing, frozen investigational vaccine/placebo should never been administered to subjects. The investigational vaccine/placebo is single use only.

All clinical trial material must be kept in an appropriate, limited-access, secure place until it is used or returned to the sponsor or designee for destruction. All sponsor-supplied vaccines/placebos must be stored under the conditions specified on the label, and remain in the original container until dispensed. A daily temperature log of the vaccine/placebo storage area must be maintained every working day. Temperature excursions must be reported to the sponsor as soon as possible and use of these vaccines/placebos requires sponsor approval. Refer to the Pharmacy Manual.

8.1.3 Dose and Regimen

Three formulations of the vaccine containing different antigen concentrations will be used, where low dose contains 2 µg, medium dose contains 5 µg, and high dose contains 10 µg of the vaccine. Each dose will be packaged and appropriately labeled in compliance with regional regulatory requirements.

Table 8.a describes the doses that will be provided to each group.

Table 8.a Sponsor-Supplied Vaccine and Placebo

Treatment Group	Dose	Treatment Description	Timing (Dose 1/Dose 2)
Arm 1	2 doses	Placebo	Day 1/Day 29
Arm 2	2 doses	PIZV 2 µg	Day 1/Day 29
Arm 3	2 doses	PIZV 5 µg	Day 1/Day 29
Arm 4	2 doses	PIZV 10 µg	Day 1/Day 29

8.2 Investigational Vaccine/Placebo Assignment and Dispensing Procedures

The investigator or investigator's designee will access the Interactive Response Technology (IRT) at Screening Visit (1A or 1B) to obtain the subject ID number.

The investigator or investigator's designee will utilize the IRT to randomize the subject at Visit 2. The investigational vaccine/placebo identification numbers to be administered will be assigned by the IRT. Details of randomization and the IRT will be covered in the IRT User Manual.

The investigational vaccine/placebo will be prepared and administered by the unblinded designee who is qualified to perform that function under applicable laws and regulations for that specific trial, according to the instructions in the Pharmacy Manual. All investigational vaccine/placebo preparation will be documented.

All doses of the investigational vaccine/placebo will be administered IM into the middle third of the deltoid muscle of the subject's arm, preferably the non-dominant arm.

The investigator or designee will be responsible for overseeing the administration of investigational vaccine/placebo to subjects enrolled in the trial according to the procedures stipulated in this trial protocol.

If vials containing the investigational vaccine/placebo are lost or damaged, the site can request a replacement from the IRT (refer to the IRT manual supplied separately). Expired trial vaccines must not be administered.

PRECAUTIONS TO BE OBSERVED IN ADMINISTERING THE TRIAL VACCINE:

Prior to vaccination, a subject must be determined to be eligible for trial vaccination and it must be clinically appropriate in the judgment of the investigator to vaccinate. Eligibility for vaccination prior to first trial vaccine administration is determined by evaluating the entry criteria outlined in this protocol (Sections 7.1 and 7.2).

Eligibility for subsequent trial vaccination is determined by following the criteria outlined in Section 7.0.

Trial vaccines should not be administered to individuals with known hypersensitivity to any component of the vaccines.

Standard immunization practices are to be observed and care should be taken to administer the injection intramuscularly. In addition, WHO recommendations to reduce anxiety and pain at the time of vaccination should be followed (91). Before administering the vaccine, the vaccination site is to be disinfected with a skin disinfectant (eg, 70% alcohol). Allow the skin to dry. DO NOT inject intravascularly.

As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccination. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

8.3 Randomization Code Creation and Storage

Randomization personnel of the IRT provider or designee will generate the randomization schedule(s). Randomization information will be stored in a secured area, accessible only by authorized personnel.

8.4 Investigational Product Blind Maintenance

This trial is an observer-blind study. The subjects, data collectors (eg, investigator), and data evaluators (eg, trial statisticians) are blinded to the material administered. The investigational product assignment will be maintained by the unblinded site staff designee.

All care must be taken to ensure that the unblinded reports and documents are shared only with unblinded personnel and properly stored in a secured area, accessible only by authorized personnel.

8.5 Unblinding Procedure

The investigational vaccine/placebo blind shall not be broken by the investigator unless information concerning the investigational vaccine/placebo is necessary for the medical treatment of the subject. In the event of a medical emergency, if possible, the medical monitor should be contacted before the investigational vaccine/placebo blind is broken to discuss the need for unblinding.

For unblinding a subject, the investigational vaccine/placebo blind can be obtained by the investigator by accessing the IRT.

The sponsor's Pharmacovigilance Department must be notified as soon as possible if the investigational vaccine/placebo blind is broken by the investigator; and if the unblinding was for medical reasons (SAE), the completed SAE form must be sent within 24 hours (ie, the site will follow the standard process for reporting of SAEs). The date, time, and reason the blind is broken must be recorded in the source document and the same information (except the time) must be recorded on the eCRF.

If any subject is unblinded, no further doses of investigational vaccine/placebo are to be administered. However, the subject will be followed up for safety and safety data will be collected according to protocol.

8.6 Accountability and Destruction of Sponsor-Supplied Vaccine/Placebo

Investigational vaccine/placebo supplies will be counted and reconciled at the site before being returned to the sponsor or designee as noted below. Sites will maintain source documents in addition to entering data in the IRT.

The investigator or designee must ensure that the sponsor-supplied vaccine/placebo is used in accordance with the approved protocol and is administered only to subjects enrolled in the trial. To document appropriate use of sponsor-supplied vaccines/placebos, the investigator must maintain records of all sponsor-supplied vaccine/placebo delivery to the site, site inventory, administration and use by each subject, and return to the sponsor or designee.

Upon receipt of sponsor-supplied vaccines/placebos, the investigator or designee must verify the contents of the shipments against the packing list. The verifier should ensure that the quantity is correct, the investigational vaccine/placebo is received within the labeled storage conditions (ie, no cold chain break has occurred during transit), and is in good condition. If quantity and conditions are acceptable, investigator or designee will acknowledge receipt of the shipment by recording in IRT.

If there are any discrepancies between the packing list versus the actual product received, the sponsor or designee must be contacted to resolve the issue. The packing list should be filed in the investigator's essential document file.

The investigator must maintain 100% accountability for all sponsor-supplied vaccines/placebos received and administered during his or her entire participation in the trial. Proper vaccine/placebo accountability includes, but is not limited to:

- Verifying that actual inventory matches documented inventory;
- Verifying that the log is completed for the vaccine/placebo used to prepare each dose;
- Verifying that all containers used are documented accurately on the log;
- Verifying that required fields are completed accurately and legibly.

If any dispensing errors or discrepancies are discovered, the sponsor must be notified immediately.

The investigator must record the current inventory of all sponsor-supplied vaccines/placebos on a sponsor-approved vaccine/placebo accountability log. The following information will be recorded at a minimum: protocol number and title, name of investigator, site identifier and number, description of sponsor-supplied vaccines/placebos, date and amount. The log should include all required information as a separate entry for each subject to whom sponsor-supplied vaccine/placebo is administered.

The investigator will be notified of any expiry date or retest date extension of clinical trial material during the trial conduct. On expiry date notification from the sponsor or designee, the site must complete all instructions outlined in the notification, including segregation of expired clinical trial material for return to the sponsor or its designee for destruction.

Prior to site closure or at appropriate intervals throughout the trial, before any clinical trial materials are returned to the sponsor or its designee for destruction, a representative from the sponsor or its designee will perform clinical trial material accountability and reconciliation. The investigator will retain a copy of the documentation regarding clinical trial material accountability, return, and/or destruction, and originals will be sent to the sponsor or designee.

The pharmacist (or designated individual) at each site will be responsible for vaccine/placebo accountability and will document receipt, use, return, or destruction of PIZV. Vaccine/placebo accountability documentation will be reviewed by the monitor during clinical monitoring visits.

9.0 TRIAL PLAN

9.1 Trial Procedures

The following sections describe the trial procedures and data to be collected. For each procedure, subjects are to be assessed by the same investigator or site personnel whenever possible. The Schedule of Trial Procedures is located in Section 2.1.

9.1.1 Informed Consent

The requirements of the informed consent are described in Section 15.2.

Informed consent must be obtained at Screening Visit (1A or 1B), prior to the subject entering into the trial and before any protocol-directed procedures are performed.

A unique subject ID number will be assigned at Screening Visit (1A or 1B) to each subject after informed consent is obtained from the appropriate coding eg, IRT. If all eligibility criteria are fulfilled, this subject ID number will be used throughout the trial. Subject ID numbers assigned to subjects who fail screening should not be reused (Section 9.1.10).

Flavivirus primed subjects who were tested at Screening Visit 1A and accepted to enter the second phase of the study (at Screening Visit 1B) do not need to sign the ICF again. Refer to Section 6.1 for more details about the staggered enrollment.

9.1.2 Demographics, Medical History, Travel History, Prior/Concomitant Medications/Vaccinations, and Blood Donation

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. Refer to Section 6.1 for more details about the staggered enrollment.

Medical history will also 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.

Medical occurrences before administration of the first dose of investigational vaccine/placebo are considered medical history.

Travel history to flavivirus endemic countries and flavivirus endemic regions of the US/US territories within 4 weeks prior to screening will be collected at Screening Visit 1A. Travel history to flavivirus endemic countries and flavivirus endemic regions of the US/US territories will be collected at each visit.

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.

Medications taken for prophylaxis are those intended to prevent the onset of AEs following vaccination. Medications taken for treatment are intended to reduce or eliminate the presence of symptoms that are present. These data must be recorded in the source documents.

Prohibited Therapies (See Section 7.2)

- Parenteral, epidural or intra-articular immunoglobulin preparation, blood products, and/or plasma derivatives within 3 months of trial vaccination.
- Immunosuppressive therapy within 3 months or systemic (eg, oral or parenteral) corticosteroid treatment within 60 days prior to trial vaccine administration.
- Other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 in this trial. Any planned vaccinations during the trial should be discussed with the investigator and if given recorded on the trial eCRFs.
- Immunostimulants within 60 days prior to trial vaccine administration.

Subjects should not donate blood during the study period.

Medical history (including corresponding medication) to be obtained will include any significant conditions or diseases that have disappeared or resolved at or prior to signing of informed consent.

All subjects will be tested for flaviviral serological status at Screening Visit (1A or 1B).

9.1.3 Documentation of Trial Entrance/Randomization

Only subjects who have signed an ICF at Screening Visit (1A or 1B), meet all of the inclusion criteria and none of the exclusion criteria, are eligible for entrance/randomization into the vaccination phase.

One single subject ID number will be assigned to each subject at Screening Visit (1A or 1B). Eligible subjects will be assigned randomly to one of the four study arms in a (1:1:1:1) ratio by a computer.

The randomization schedule will be created and controlled by the IRT provider. The randomization specification will be approved by the sponsor's trial statistician or designee.

If the subject is found to be ineligible for randomization/trial phase, the investigator should record the primary reason for failure on the subject enrollment screening log.

9.1.4 Physical Examination

Physical examinations must be performed by a qualified health professional in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log. Complete physical examination, including height and weight, will be performed at Screening Visit(s) (Visit 1A and/or Visit 1B) according to the investigator's standard practice. Measurement

of height and weight is only required at Screening Visit(s) (Visit 1A and/or Visit 1B). BMI will be calculated using standard BMI calculator.

Additional symptom-directed physical examinations may be performed at following visits if deemed necessary or indicated by review of the subject's medical history, and should assess clinically significant changes from the baseline examination.

The following should be documented in the subject's source document:

- The findings of complete physical examinations,
- the findings of symptom-directed physical examinations,
- if symptom-directed physical examinations were not required.

Findings consistent with the definition of AEs or SAEs (see Section 10.0) must be transcribed into the eCRF.

9.1.5 Vital Signs

Vital signs include (however, not limited to) systolic/diastolic blood pressure, pulse rate, respiratory rate, and temperature. Follow standard of care for trial population and operational feasibility.

Vital signs must be within normal limits (ie, below Grade 1 as specified in the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers, see Appendix E).

In the event of abnormal heart rate or blood pressure due to physiological variation or activity, the subject may rest for 10 minutes in a quiet room, and then blood pressure and/or heart rate may be re-measured. Repeated vital signs may be used to determine eligibility.

9.1.6 Blood Sample and Urine Sample Collection

Blood samples will be collected at each site visit: ie, at Screening Visit(s) (Visit 1A and/or Visit 1B) for flavivirus serostatus determination (see Section 9.1.6.1) and eligibility screening tests (including pregnancy testing) (see Section 9.1.6.2), at Visits 3 and 5 for routine safety laboratory testing (see Section 9.1.6.3), 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 (see Section 9.1.6.4). 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. Blood should be taken from the subject using an aseptic venipuncture technique. Blood samples will be sent to the central laboratory.

Urine samples will be collected for all subjects at Screening Visit(s) (Visit 1A and/or Visit 1B) for eligibility screening (see Section 9.1.6.2), and at Visits 3 and 5 for routine safety laboratory testing

(see Section 9.1.6.3); and for women of childbearing potential at Visit 2 and 4 before each investigational vaccine/placebo administration for pregnancy testing (see Section 9.1.9). 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.

All samples will be collected in accordance with acceptable laboratory procedures. All samples will be processed and stored at the trial site as described in the provided Laboratory Manual.

9.1.6.1 Flavivirus Serostatus Determination

All subjects who sign the ICF will be tested for flavivirus serostatus determination at Screening Visit (1A or 1B). For Screening, the subject's serostatus relevant to major flavivirus(es) will be evaluated (including but not limited to Dengue, ZIKA, West Nile, Japanese Encephalitis).

Blood samples for flavivirus serostatus determination will be approximately 10 mL. Refer to the Serology Plan, available on file.

Each subject will be tested only once for flavivirus serostatus determination.

The first phase of the study will be aimed at enrolling flavivirus naïve subjects only, while the second phase of the study will be aimed at enrolling flavivirus primed subjects only. Flavivirus primed subjects will not be enrolled (randomized) during the first phase of the study (ie, when identified at Screening Visit 1A) and will be invited to come back for the second phase of the study. Refer to Section 6.1 for more details about the staggered enrollment.

9.1.6.2 Eligibility Screening Tests

Screening laboratory tests that will be performed on blood and urine samples at Screening Visit(s) (Visit 1A and/or Visit 1B) are outlined in Table 9.a.

In order for the subjects to be enrolled, they must have laboratory values within normal limits or not be above Grade 1 as defined in the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers (see Appendix E) within 15 days before enrollment. If laboratory screening tests are out of acceptable range, repeat of screening tests is permitted once, provided there is an alternative explanation for the out of range value.

Should the respective repeated test results be available within the initial screening period, no new subject ID numbers will be required; the repeated screening tests will be recorded in the appropriate CRF (unscheduled lab form). Should the respective repeated test results not be available within the initial screening period, the subject would need to be screen failed and all screening procedures and tests would need to be repeated with a new subject ID number.

Blood samples for screening laboratory testing will be approximately 10 mL.

Subjects who are tested flavivirus primed at Screening Visit 1A (during the first phase of the study) and accept to enter the second phase of the study will undergo an additional Screening Visit (Visit 1B) during which screening laboratory testing will be tested again. Refer to Section 6.1 for more details about the staggered enrollment.

Table 9.a Clinical Laboratory Assessments

Sample		Test
Blood	Hematology	Hemoglobin Platelet count White blood cell count Total neutrophils Eosinophils Lymphocytes PT (prothrombin time) APTT (activated partial thromboplastin time) Fibrinogen
	Biochemistry	Blood urea nitrogen Creatinine AST (aspartate transaminase) ALT (alanine transaminase) Alkaline phosphatase Sodium Potassium Chloride Bicarbonate
	Pregnancy test	β-hCG blood test (only at Screening Visit(s) Visit 1A and/or Visit 1 B)
Urine	Urinalysis	Protein Glucose Blood (microscopic) Red blood cells per high power field

9.1.6.3 Safety Laboratory Testing

Routine safety laboratory parameters that will be performed on blood and urine samples at Visit 3 (Day 8) and Visit 5 (Day 36) are outlined in Table 9.a.

Each blood sample will be approximately 10 mL.

9.1.6.4 Immunogenicity Assessments

Subjects in all study arms will undergo blood sampling for serological immunogenicity testing at Visit 2 (Day 1), Visit 4 (Day 29), Visit 6 (Day 57), Visit 8 (Day 211), and (for subjects who will be randomized to the placebo group or the PIZV dosing group that will be selected for further development) Visit 9 (Day 393) and Visit 11 (Day 757). Should a blood sampling be performed during a vaccination visit, this sampling must occur before the administration of the investigational vaccine or placebo. Refer to the Serology Plan, available on file.

Each blood sample for immunogenicity assessments will be approximately 60 mL. Some of it will be used for further development and characterization of assays if subjects agree and sign the ICF.

9.1.7 Safety Assessments

During the trial, safety assessments will include collection and recording of solicited local (injection site) and systemic AEs (including fever), unsolicited AEs (serious and non-serious), and new medical conditions (neurological and neuroinflammatory disorders) with onset after the first vaccination. Refer to Section 10.1 for safety definitions. Details on collection and reporting of AEs are in Section 10.5. Refer to Section 9.3.4 for details about the diary cards distribution, review and collection processes.

9.1.8 Contraception and Avoidance of Sexually Transmitted Disease Guidance

Subjects will be provided with information on acceptable methods of contraception and of protection against sexually transmitted diseases.

Female subject of childbearing potential and sexually active will have to use an "acceptable contraceptive method" from trial entry through 2 months after the last dose of trial vaccine, and will be advised not to donate ova during this period.

Furthermore, to avoid sexual transmission of ZIKV from natural exposure, all enrolled sexually active subjects (both male and female) will have to use latex condoms correctly and consistently even if other contraceptive measures are used from signing the ICF through the end of the trial. Male subjects must be advised not to donate sperm during this period.

Refer to Section 7.2 for more details.

9.1.9 Pregnancy

In women of childbearing potential, a serum pregnancy testing will be performed at Screening Visit(s) (Visit 1A and/or Visit 1B), and a urine pregnancy testing will be performed at Visit 2 (Day 1) before randomization (and thereby before investigational vaccine/placebo dose 1 administration) for confirmation of eligibility, as well as at Visit 4 (Day 29) before investigational vaccine/placebo dose 2 administration.

Serum pregnancy tests will be conducted in the central laboratory (and will be part of the eligibility screening tests) and urine pregnancy tests will be performed at the study site using kits provided by the sponsor.

Subjects must have a negative serum β-hCG pregnancy test at screening and a negative urine β-hCG pregnancy test prior to receiving any dose of investigational vaccine/placebo.

To ensure subject safety and the safety of the unborn child, each pregnancy in a subject having received an investigational vaccine/placebo must be reported to the sponsor within 24 hours of the site learning of its occurrence. If the subject becomes pregnant during the trial, she will not receive any further doses of any sponsor-supplied investigational vaccine/placebo. The pregnancy must be followed to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. This follow-up should occur even if the intended duration of safety follow-up for the trial has ended.

Any pregnancy occurring following investigational vaccine/placebo administration should be reported immediately, using a pregnancy notification form, to the contact listed in the Investigator Site File.

Should the pregnancy occur after administration of a blinded investigational vaccine/placebo, the investigator must inform the subject of their right to receive information on the study vaccine/placebo administered. If the subject chooses to receive unblinded information, the individual blind should be broken by the investigator and procedures must be followed, as described in Section 8.5.

9.1.10 Documentation of Subjects who are not Randomized

Investigators must account for all subjects who sign an ICF. If the subject is found to be not eligible at this visit, the investigator should complete the eCRF. The IRT supplier should be contacted as a notification of non-randomization.

The primary reason for non-randomization is recorded in the eCRF using the following categories:

- Screen failure
    - did not meet one or more inclusion criteria
    - did meet one or more exclusion criteria
    - tested flavivirus naïve at Screening Visit 1B (during the second phase of the study)
    - tested flavivirus primed at Screening Visit 1A (during the first phase of the study) and did not come back for the second phase of the study
    - with a non-determinant screening test result
- Withdrawal by subject,
- Trial terminated by sponsor.

A subject may be a temporary screen failure under the following circumstances: due to temporary conditions such as receipt of immunosuppressive or immunomodulatory therapies, blood products, immunoglobulins, or vaccines, in the respective prohibited periods (see exclusion criteria 7, 13 or 14), or in the event that a subject has an indeterminate pregnancy test result or has not used acceptable contraceptive methods in the period defined in the protocol, prior to the start of the study (exclusion criteria 19). Subjects screen failed for the above-mentioned reasons may be rescreened once they cease to meet the respective exclusion criteria. Should the respective repeated test results be available within the initial screening period, no new subject ID numbers will be required; the repeated screening tests will be recorded in the appropriate CRF (unscheduled lab form). Should the respective repeated test results not be available within the initial screening period, the subject would need to be screen failed and all screening procedures and tests would need to be repeated with a new subject ID number.

Subject ID numbers assigned to subjects who fail screening should not be reused.

9.2 Monitoring Subject Treatment Compliance

The investigator records all injections of investigational vaccine/placebo given to the subject in the eCRF.

9.3 Schedule of Observations and Procedures

The schedule for all trial-related procedures for all evaluations is shown in Section 2.1. Assessments should be completed at the designated visit(s)/time point(s).

9.3.1 Screening Procedures (Screening Visit 1A/ Screening Visit 1B)

Refer to Section 6.1 for more details about the staggered enrollment.

The following screening procedures will be performed at Screening Visit(s) (Visit 1A and/or Visit 1B):

1. Confirm informed consent, and complete and collect ICF (see Section 9.1.1). Before performing any other trial procedure, the signed ICF needs to be obtained. Only applicable for newly screened subjects.
2. Assign subject ID number (see Section 6.1 for more details about the staggered enrollment).
3. Assess eligibility criteria (see Sections 7.1 and 7.2).
4. Collect demographics (see Section 9.1.2). Only applicable for newly screened subjects.
5. Collect medical history, travel history (to flavivirus endemic countries and flavivirus endemic regions of the US/US territories), and prior/concomitant medications/vaccinations (see Section 9.1.2).
6. Perform “complete” physical examination (see Section 9.1.4).
7. Check vital signs (see Section 9.1.5).
8. Collect blood sample for flavivirus serostatus (only applicable for newly screened subjects, see Section 9.1.6.1) and eligibility screening tests (hematology, biochemistry and pregnancy testing, see Section 9.1.6.2).
9. Collect urine sample for eligibility screening urinalysis (for all subjects, see Section 9.1.6.2).
10. Provide contraception and avoidance of sexually transmitted disease guidance (see Section 9.1.8).

9.3.2 Pre-Vaccination Procedures (Day 1 and Day 29)

The following procedures will be performed at Visit 2 (Day 1) and Visit 4 (Day 29):

1. Collect medical history, travel history (to flavivirus endemic countries and flavivirus endemic regions of the US/US territories), and concomitant medications/vaccinations (see Section 9.1.2).
2. Perform symptom-directed physical examination (if deemed necessary, see Section 9.1.4).

3. Assess eligibility criteria (see Sections 7.1 and 7.2) at Visit 2 (Day 1).
4. Check vital signs (see Section 9.1.5).
5. Perform a urine pregnancy test (if the subject is a woman of childbearing potential, see Section 9.1.9).
6. Randomize subject (only at Visit 2 on Day 1) (see Section 9.1.3).
7. Collect blood sample for immunogenicity assessment and assay development (see Section 9.1.6.4).

9.3.3 Vaccination Procedures (Day 1 and Day 29)

The following procedures will be performed at Visit 2 (Day 1) and Visit 4 (Day 29):

1. Check contraindications to vaccination and criteria for delay of vaccination (see Sections 7.2 and 7.3).
2. Prepare the investigational vaccine/placebo according to the Pharmacy Manual (see Section 8.2).
3. Administer the investigational vaccine or placebo (see Section 8.2).

9.3.4 Post Vaccination Procedures (Day 1 and Day 29)

The following post-vaccination procedures will be performed at Visit 2 (Day 1) and Visit 4 (Day 29):

1. After vaccination, the subject will be observed for at least 30 minutes including observation for immediate reactions and body temperature measurement. Information should be recorded in the eCRF as immediate reactions. The investigator or delegate will take the opportunity to remind the subject how to measure solicited AEs and body temperature as part of this observation period. All safety data will be collected in the subject's source documents.
2. Provide the subject with a diary card, and train the subject on how to use it.

   Careful training of the subject on how to record concomitant medications, how to measure solicited AEs and body temperature, how to complete the diary card and how often to complete the diary card. Training should be directed at the individual(s) who will perform the measurements of solicited AEs and those who will enter the information into the diary card. This individual may or may not be the subject, but if a person other than the subject enters information into the diary card, this person's identity must be documented in the trial file and this person must receive training on the diary card. Training of the subject on how to measure an injection site AE should be performed while the subject is under observation after vaccination.

   Diary card instructions must include the following: The subject must understand that timely completion of the diary card on a daily basis is a critical component of trial participation. The subject should also be instructed to write clearly and to complete the diary card in pen. Any corrections to the diary card that are performed by the person completing the diary card should

include a single strikethrough line with a brief explanation for any change and be initialed and dated.

Please note:

Diary cards will be the only source document allowed for remote collection of solicited local and systemic AEs (including body temperature measurements). The following additional rules apply to the documentation of safety information collected by diary card:

- Diary cards should be reviewed with the subject.
- No corrections or additions to the diary card will be allowed after it is reviewed with the investigator/designee.
- Any data that are identified as implausible or incorrect, and confirmed by the subject to be a transcription error should be corrected by the subject on the diary card (the correction should include a single strikethrough line and should be initialed and dated by the subject).
- Any blank or illegible fields on the diary card not otherwise corrected as above will be missing in the eCRF.
- The site must enter all readable entries on the diary card into the eCRF.
- Any newly described solicited safety information should be added to the diary card by the subject and initialed and dated. Any new unsolicited safety information would be recorded in the subject source document as a verbally reported event and therefore captured as an AE and recorded in the AE eCRF.
- Starting on the day of vaccine/placebo administration, the subject will check for specific types of events at the injection site (solicited local AEs), any specific generalized symptoms (solicited systemic AEs), body temperature (preferably oral), any other symptoms or change in the subject's health status, and any medications taken (excluding vitamins and minerals). These solicited AEs and body temperature will be recorded in the diary. Assessments should preferably take place in the evening at day's end using the same route of measurement every day.
- Temperature measurement is to be performed using the thermometer provided by the site. If the subject feels unusually hot or cold during the day, the subject should check his/her temperature. The highest body temperature observed that day should be recorded on the diary card.
- The measurement of solicited local AEs (erythema, swelling, and induration) is to be performed using the ruler provided by the site. If multiple measurements are taken during the day, the highest measured value should be recorded on the diary card.
- The collection on the diary card of body temperature, solicited local AEs, and solicited systemic AEs will continue for a total of 7 days following each vaccine/placebo administration. The collection on the diary card of unsolicited AEs and medications will continue for 28 days following each vaccine/placebo administration.

- The healthcare professional reviewing the data on the diary cards will discuss the AEs (if any) reported by the subject and will determine if any additional diagnoses and/or AEs are present and/or concomitant medications have been used.

3. At Visit 4 (Day 29), review the previous diary card with the subject and collect it (see Section 9.3.4).
4. Provide contraception and avoidance of sexually transmitted disease guidance (see Section 9.1.8).
5. Provide the subject with a written reminder of the next planned trial activity. The subject will be reminded to complete the diary card daily and to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious. All contact details will be provided to the subject.
6. Schedule the next trial clinic visit with the subject.

9.3.5 Clinic Visits after Vaccination (Day 8, Day 36, Day 57, Day 211, and Day 393)

The following procedures will be performed at Visit 3 (Day 8), Visit 5 (Day 36), Visit 6 (Day 57), and for subjects randomized to the placebo group or the PIZV dosing group that was selected for further development, Visit 8 (Day 211) and Visit 9 (Day 393):

1. Collect travel history (to flavivirus endemic countries and flavivirus endemic regions of the US/US territories) and concomitant medications/vaccinations (see Section 9.1.2).
2. Perform symptom-directed physical examination (if deemed necessary, see Section 9.1.4).
3. Check vital signs (see Section 9.1.5).
4. Collect blood sample for routine safety laboratory testing (only at Visit 3 on Day 8 and Visit 5 on Day 36, see Section 9.1.6.3) and immunogenicity assessment and assay development (only at Visit 6 on Day 57, Visit 8 (Day 211), and Visit 9 (Day 393); see Section 9.1.6.4).
5. Collect urine sample for routine safety laboratory testing (urinalysis; only at Visit 3 on Day 8 and Visit 5 on Day 36; see Section 9.1.6.3).
6. Review the previous diary card with the subject and collect it (see Section 9.3.4).
7. At Visit 3 (Day 8) and Visit 5 (Day 36), provide the subject with a new diary card, and remind the subject on how to use it (see Section 9.3.4).
8. Provide contraception and avoidance of sexually transmitted disease guidance (see Section 9.1.8).
9. Provide the subject with a written reminder of the next planned trial activity. The subject will be reminded (only at Visit 3 on Day 8 and Visit 5 on Day 36) to complete the diary card daily. The subject will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical

condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious. All contact details will be provided to the subject.

10. Schedule the next trial clinic visit with the subject.

9.3.6 Phone Contacts (Day 133 and Day 575)

A phone contact will be made on Day 133 (Visit 7) and Day 575 (Visit 10) with each subject to collect SAEs that may have occurred since Visit 6 (Day 57) and Visit 9 (Day 393), respectively, and to remind the subject of the next planned (end of) trial activity. The subject will also be reminded to contact the site if there are any questions, and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious. All contact details will be provided to the subject. The subject should be reminded to contact the site via the telephone number provided in the ICF to discuss medical questions.

Travel history (to flavivirus endemic countries and flavivirus endemic regions of the US/US territories) will be collected.

If the subject wishes to describe safety information, this information should only be collected by a trained healthcare professional at the site, and the safety data described must be written down in source documents.

The subject will be interviewed according to a script, and information relating to SAEs and/or AEs leading to trial or vaccine withdrawal and concomitant medications or vaccinations associated with those events will be collected. All safety information described by the subject must be written down in a designated location within the source documents and not written on the script used for the telephone call.

The site should schedule the next clinic visit with the subject, as appropriate.

9.3.7 Final Visit (Day 211 or Day 757)

The Final Visit will be performed at Visit 8 (Day 211) or, for subjects randomized to the placebo group or the PIZV dosing group that was selected for further development, Visit 11 at Day 757 (24 months post dose 2). If a subject terminates earlier, Final Visit procedures should be performed, if possible.

The following procedures will be performed at Visit 8 (Day 211) or Visit 11 (Day 757):

1. Collect travel history (to flavivirus endemic countries and flavivirus endemic regions of the US/US territories) and concomitant medications/vaccinations (see Section 9.1.2).
2. Perform symptom-directed physical examination (if deemed necessary, see Section 9.1.4).
3. Check vital signs (see Section 9.1.5).
4. Collect blood sample for immunogenicity assessment and assay development (see Section 9.1.6.4).

5. For all subjects having received the investigational vaccine or the placebo, the investigator must complete the End of Trial eCRF page.

9.3.8 Post-Trial Care

No post-trial care will be provided.

9.4 Biological Sample Retention and Destruction

In this trial, specimens for immune response testing will be collected as described in Section 9.1.6.4. After blood draw and serum processing, the serum samples will be preserved and retained at a central storage location that was contracted by the sponsor for this purpose for up to but not longer than 20 years or as required by applicable law. The sponsor has put into place a system to protect the subjects' personal information to ensure optimal confidentiality and defined standard processes for sample and data collection, storage, analysis, and destruction.

Serum samples will be used for the analyses defined in this protocol, but can also, with permission from the subject, be used to assess, improve or develop tests related to the disease(s) or the vaccine under trial that will allow more reliable measurement of the response to the vaccine.

10.0 ADVERSE EVENTS

10.1 Definitions

10.1.1 Adverse Events (AEs)

An AE is defined as any untoward medical occurrence in a clinical investigation subject administered a trial vaccine; it does not necessarily have to have a causal relationship with trial vaccine administration.

An AE can therefore be any unfavorable and unintended sign (eg, a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the administration of a trial vaccine whether or not it is considered related to the trial vaccine.

AEs will be graded by the investigator in the following manner:

Mild	Grade 1	• Awareness of symptoms that are easily tolerated, causing minimal discomfort and not interfering with everyday activities. Relieved with or without symptomatic treatment.
Moderate	Grade 2	• Sufficient discomfort is present to cause interference with normal activity. Only partially relieved with symptomatic treatment.
Severe	Grade 3	• Extreme distress, causing significant impairment of functioning or incapacitation. Prevents normal everyday activities. Not relieved with symptomatic treatment

10.1.2 Solicited Adverse Events

The occurrence of selected indicators of safety (Table 10.a) will be measured/collected for 7 days after each dose (including the days of vaccine administration) and will be recorded on the "Local and Systemic AEs" eCRF as applicable. These will be summarized in the final report under the category "solicited adverse events" to differentiate them from other AEs which were not solicited. Any solicited local or systemic AE observed as continuing beyond 7 days after vaccination will be recorded as an unsolicited AE on the Adverse Event eCRF. Any solicited local (injection site) or systemic AE observed as continuing beyond 7 days following each trial vaccination will be additionally recorded on the Adverse Event eCRF for follow-up. For these persistent/prolonged solicited AEs, the end date will be captured on the Adverse Event eCRF to permit a separate analysis from the unsolicited AEs.

Table 10.a 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 (92)).

The intensity of solicited safety parameters will be assessed as described in Table 10.b.

Table 10.b Solicited Safety Parameters

Adverse Event	Intensity grade	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 (Brighton Case Definition (92)).

10.1.3 Serious Adverse Events (SAEs)

An SAE is defined as any untoward medical occurrence that:

1. Results in DEATH.
2. Is LIFE THREATENING.
    - The term "life threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
3. Requires inpatient HOSPITALIZATION or prolongation of existing hospitalization.
4. Results in persistent or significant DISABILITY/INCAPACITY.
5. Leads to a CONGENITAL ANOMALY/BIRTH DEFECT in the offspring of a subject.
6. Is an IMPORTANT MEDICAL EVENT that satisfies any of the following:
    - May require intervention to prevent items 1 through 5 above.
    - May expose the subject to danger, even though the event is not immediately life threatening or fatal or does not result in hospitalization.

10.2 Causality of AEs

Relatedness (causality) to vaccine will also be assessed by the investigator. The relationship of each AE, including solicited systemic AEs (solicited local AEs are considered as related) to trial vaccine(s) will be assessed using the following categories:

Related:	There is suspicion that there is a relationship between the trial vaccine and the AE (without determining the extent of probability); there is a reasonable possibility that the trial vaccine contributed to the AE.
Not Related:	There is no suspicion that there is a relationship between the trial vaccine and the AE; there are other more likely causes and administration of the trial vaccine is not suspected to have contributed to the AE.

10.2.1 Relationship to Trial Procedures

Relationship (causality) to trial procedures should be determined for all AEs.

The relationship should be assessed as "Yes" if the investigator considers that there is reasonable possibility that an event is due to a trial procedure. Otherwise, the relationship should be assessed as "No".

10.2.2 Outcome of AEs

Resolved:	The subject has fully recovered from the event or the condition has returned to the level observed at baseline
Resolving:	The event is improving but the subject is still not fully recovered
Not resolved:	The event is ongoing at the time of reporting and the subject has still not recovered
Resolved with *sequelae*:	As a result of the AE, the subject suffered persistent and significant disability/incapacity (eg, became blind, deaf or paralyzed)
Fatal:	The subject died due to the event. If the subject died due to other circumstances than the event, the outcome of the event per se should be stated otherwise (eg, Not Resolved or Resolving)
Unknown:	If outcome is not known or not reported.

10.3 Additional Points to Consider for AEs

An untoward occurrence generally may:

- Indicate a new diagnosis or unexpected worsening of a pre-existing condition. Intermittent events for pre-existing conditions or underlying disease should not be considered as AEs.
- Necessitate therapeutic intervention.
- Require an invasive diagnostic procedure.
- Require vaccine discontinuation or a change in concomitant medication.
- Be considered unfavorable by the investigator for any reason.

Diagnoses *vs* signs and symptoms:

- Each event should be recorded to represent a single diagnosis. Accompanying signs (including abnormal laboratory values) or symptoms should NOT be recorded as additional AEs. If a diagnosis is unknown, signs or symptoms should be recorded appropriately as AEs.

Worsening of AEs:

- If the subject experiences a worsening or complication of an AE after starting administration of the trial vaccine, the worsening or complication should be recorded as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").
- If the subject experiences a worsening or complication of an AE after any change in trial vaccine, the worsening or complication should be recorded as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

Changes in severity of AEs:

- If the subject experiences changes in severity of an AE, the event should be captured once with the maximum severity recorded.

Preplanned surgeries or procedures:

- Preplanned procedures (surgeries or therapies) that were scheduled prior to signing of informed consent are not considered AEs. Complications resulting from any planned surgery should be reported as AEs.

Elective surgeries or procedures:

- Elective procedures performed where there is no change in the subject's medical condition should not be recorded as AEs; however, these procedures should be documented in the subject's source documents. Complications resulting from an elective surgery should be reported as AEs.

Medical occurrences emerging during the time between signing of informed consent and the first administration of investigational vaccine/placebo will be recorded on the medical history eCRF page. However, if such medical occurrence is assessed as 'related to a study procedure' this should be reported as an AE 'related to study procedure' in the eCRF.

10.4 Procedures

10.4.1 Collection and Reporting of AEs

All AEs, whether considered related with the use of the investigational vaccine/placebo or not, must be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report should be supplied, if possible. All findings must be reported on an AE eCRF and on the SAE form, if necessary (see Section 10.4.2). All findings in subjects experiencing AEs must also be reported in the subject's source documents. Any unsolicited AEs will be collected for 28 days after each vaccine/placebo administration (ie, through trial Day 28 and trial Day 56) via diary cards. AEs leading to withdrawal or discontinuation will be collected throughout the trial.

The following information will be documented for each event:

- Reported term for the Adverse Event,
- Start and end date,
- Serious (Y/N),
- Severity,
- Investigator's opinion of the causality (relationship) between the event and administration of trial vaccine(s) ("related" or "not related"),

- Investigator's opinion of the causality (relationship) to trial procedure(s), including the details of the suspected procedure,
- Action taken with the trial treatment (trial vaccine),
- Outcome of event.

10.4.2 Collection and Reporting of Solicited AEs

The occurrence of selected indicators of safety will be collected on diary cards by the subjects for 7 days following each investigational vaccine/placebo administration (ie, the day of vaccination +6 subsequent days) and will be recorded on the "Local and Systemic AE" eCRF, as applicable. These will be summarized in the final report under the category "solicited adverse events" to differentiate them from unsolicited AEs. Any solicited local (injection site) or systemic AE observed as continuing beyond 7 days following each trial vaccination will be additionally recorded on the Adverse Event eCRF for follow-up. For these persistent/prolonged solicited AEs, the end date will be captured on the Adverse Event eCRF to permit a separate analysis from the unsolicited AEs.

Any solicited AE that meets any of the following criteria must be entered as an AE on the Adverse Event eCRF.

- Solicited local or systemic AEs that lead the subject to withdraw from the trial.
- Solicited local or systemic AEs that lead to the subject being withdrawn from the trial or to discontinuation of vaccination by the investigator.
- Solicited local and systemic AEs that otherwise meet the definition of an SAE (see Section 10.1.2).

10.4.3 Collection and Reporting of SAEs

Collection of SAEs will commence from the time that the subject is first administered the investigational vaccine/placebo (Day 1). Routine collection of SAEs will continue up to the end of the trial at Visit 8 (Day 211) or Visit 11 (Day 757) for subjects who were randomized to the placebo group or the PIZV dosing group that was selected for further development.

SAEs should be reported according to the following procedure:

A sponsor SAE form must be completed, in English, and signed by the investigator immediately or within 24 hours of first onset or notification of the event. The information should be completed as fully as possible but contain, at a minimum:

- A short description of the event and the reason why the event is categorized as serious.
- Subject identification number.
- Investigator's name.
- Name of the trial vaccine(s) – in a blinded way at all times, unless there is a valid reason to unblind.

- Causality assessment.

The SAE form should be transmitted within 24 hours to the attention of the contact(s) in the list provided to each site.

Note: For this study, SAE reporting will be performed via eCRF. Only if the Electronic Data Capture system (EDC) is unavailable, a paper sponsor SAE form should be completed, signed by the investigator and transmitted within 24 hours. The SAE must be entered into the eCRF once access to the EDC is restored.

10.5 Follow-up Procedures

10.5.1 AEs

All AEs will be monitored until resolution or a stable status is reached or until a formal diagnosis can be made or until the end of the trial, whichever occurs first.

10.5.2 SAEs

If information not available at the time of the first report becomes available at a later date, the investigator should complete a follow-up SAE form or provide other written documentation and fax it immediately within 24 hours of receipt. Copies of any relevant data from the hospital notes (eg, laboratory tests, discharge summary, postmortem results) should be sent to the sponsor.

All SAEs should be followed up until resolution or permanent outcome of the event(s) or until they are otherwise explained (outside of the trial, after Visit 11 at Day 757, whichever applicable). The timelines and procedure for follow-up reports are the same as those for the initial report.

10.5.3 Safety Reporting to Investigators, IRBs or IECs, and Regulatory Authorities

The sponsor or designee will be responsible for the reporting of all suspected unexpected serious adverse reactions (SUSARs) and any other applicable SAEs to regulatory authorities, investigators and IRBs or IECs, as applicable, in accordance with national regulations in the countries where the trial is conducted. Relative to the first awareness of the event by/or further provision to the sponsor or sponsor's designee, SUSARs will be submitted within 7 days for fatal and life-threatening events and 15 days for other SUSARs, unless otherwise required by national regulations. The sponsor will also prepare an expedited report for other safety issues where these might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in the trial vaccine administration or in the overall conduct of the trial. The investigational site also will forward a copy of all expedited reports to their IRB or IEC in accordance with national regulations.

10.5.4 Post-Trial Events

Any SAE that occurs outside of the protocol-specified observation period or after the end of the trial but considered to be caused by the trial vaccine(s) must be reported to the sponsor. These SAEs will be processed by the sponsor's Pharmacovigilance Department. Instructions for how to submit these SAEs will be provided in a handout in the Investigator Site File.

11.0 TRIAL-SPECIFIC REQUIREMENTS

11.1 Data Monitoring Committee Guidelines for Possible Study Pause

The external DMC will review safety data on an ongoing basis (refer to Section 11.2.1). More specifically, when the following set of AEs are reported, the external DMC will review all relevant safety data within 48 hours and consider a possible pause of the study, if it deems necessary:

- Two or more subjects develop the same grade 3 solicited AE starting within 7 days of vaccination
- Two or more subjects develop the same grade 3 unsolicited AE within 28 days of vaccination
- Any SAE
- Any unexpected, serious, or unanticipated risk to the subjects enrolled.

11.2 Trial-Specific Committees

11.2.1 External Data Monitoring Committee

An external DMC will have oversight of this trial. The external DMC functions at a program level and further information is available in the external DMC Charter. The external DMC may review the data in an unblinded manner; however, the study team will remain blinded. The external DMC will receive predefined standard tables and listings of exposure and demographic data, solicited and unsolicited AEs, SAEs, new medical conditions (neurological and neuroinflammatory disorders) with onset after the first vaccination, and laboratory data obtained at specific data lock points for review. Any additional data requested by the external DMC will also be provided as needed.

The external DMC will consist of 5 independent members and an independent non-voting statistician. External DMC meetings will consist of open and closed face-to-face meetings or teleconference calls. The type and frequency of scheduled meetings will depend on the subject enrollment and safety event rates. Unscheduled ad hoc meetings will occur at any time consultation is requested by the Pharmacovigilance Study Team. The recommendations will be communicated by the Chair of the external DMC to the Head of Pharmacovigilance in a timely manner. Takeda Vaccines will notify investigators and the appropriate regulatory authorities if the study is modified, placed on hold, completed, or closed.

12.0 DATA HANDLING AND RECORDKEEPING

The full details of procedures for data handling will be documented in the Data Management Plan. AEs, medical history, and concurrent conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA – System Organ Class [SOC], High Level Group Term [HLGT], High Level Term [HLT], Lowest Level Term [LLT], Preferred Term [PT], and their corresponding descriptive terms). Drugs will be coded using the WHO Drug Dictionary.

12.1 Electronic CRFs (eCRF)

Completed eCRFs are required for each subject who provides a signed ICF.

The sponsor or its designee will supply investigative sites with access to eCRFs. The sponsor will make arrangements to train appropriate site staff in the use of the eCRF. These forms are used to transmit the information collected in the performance of this trial to the sponsor and regulatory authorities. eCRFs must be completed in English.

After completion of the entry process, computer logic checks will be run to identify items, such as inconsistent dates, missing data, and questionable values. Queries may be issued by sponsor personnel (or designees) and will be answered by the site.

Corrections to eCRFs are recorded in an audit trail that captures the old information, the new information, identification of the person making the correction, the date the correction was made, and the reason for change.

The principal investigator or designee must review the eCRFs for completeness and accuracy and must sign and date the appropriate eCRFs as indicated. Furthermore, the investigator must retain full responsibility for the accuracy and authenticity of all data entered on the eCRFs.

eCRFs will be reviewed for completeness and acceptability at the trial site during periodic visits by trial monitors. The sponsor or its designee will be permitted to review the subject's medical and hospital records pertinent to the trial to ensure accuracy of the eCRFs. The completed eCRFs are the sole property of the sponsor and should not be made available in any form to third parties, except for authorized representatives of appropriate governmental health or regulatory authorities, without written permission of the sponsor.

12.2 Record Retention

The investigator agrees to keep the records of the eCRF and those documents that include (but are not limited to) the trial-specific documents, the identification log of all participating subjects, source documents. Temporary media such as thermal sensitive paper should be copied and certified, source worksheets, all original signed and dated ICFs, subject authorization forms regarding the use of personal health information (if separate from the ICFs), electronic copy of eCRFs, including the audit trail, and detailed records of vaccine disposition to enable evaluations or audits from regulatory authorities, the sponsor or its designees. Furthermore, ICH E6 (2) Section 4.9.5 requires the investigator to retain essential documents specified in ICH E6 (2) Section 8 until at least 2 years after the last approval of a marketing application for a specified vaccine indication being investigated or, if an application is not approved, until at least 2 years

after the investigation is discontinued and regulatory authorities are notified. In addition, ICH E6 (2) Section 4.9.5 states that the trial records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the Clinical Study Site Agreement between the investigator and sponsor.

Refer to the Clinical Study Site Agreement for the sponsor's requirements on record retention. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

13.0 STATISTICAL METHODS

13.1 Statistical and Analytical Plans

A statistical analysis plan (SAP) will be prepared and finalized prior to unblinding of subject's treatment assignment. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all trial objectives.

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.

13.1.1 Analysis Sets

- *Safety Set*: The Safety Set will consist of all randomized subjects who received at least one dose of the investigational vaccine/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 include all subjects in the FAS who have no major protocol violations. The major protocol violation criteria will be defined as part of the blinded data review prior to the unblinding of subject's investigational vaccine/placebo assignment. The categories of major protocol violations include:

a) not meeting selected entry criteria,

b) receiving a wrong investigational vaccine/placebo,

c) receiving prohibited therapies, and

d) other major protocol violations that may be identified during blinded data reviews.

All summaries and analyses of safety data will be based on subjects in the Safety Set.

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

13.1.2 Analysis of Demographics and Other Baseline Characteristics

Summaries of age, gender, race, and other baseline characteristics will be presented by formulation arm.

13.1.3 Immunogenicity Analysis

- *Seropositive subjects*: Subjects with detectable serum antibodies (tested positive at or above limit of detection, LOD) as measured by the neutralization assay.

- *Seronegative subjects*: Subjects with no detectable serum antibodies (test results are below 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 with different fold increases in post-vaccination antibodies from baseline, as measured by the neutralization assay.

Descriptive statistics for the primary and secondary immunogenicity endpoints, including estimates and 95% confidence intervals (95% CI) for GMT, SPR and SCR, will be provided by time point (28 days post dose 1, 28 days post dose 2, and 6, 12, and 24 months post dose 2 in applicable groups) and by formulation arm.

Point estimates and 95% CI for ratios in GMT and differences in SPR and SCR will be provided for each pair of active study arms (ie, low, medium and high doses) to aid a single vaccine dose level selection.

Immunogenicity summaries and analyses will be provided by dose group overall, as well as by baseline flavivirus serostatus.

13.1.4 Safety Analysis

Reactogenicity will be assessed for 7 days following each dose (including day of vaccine/placebo administration) via daily collection of solicited AEs, including local reactions (injection site: pain, erythema, swelling, and induration) and systemic AEs of headache, fatigue, malaise, arthralgia and myalgia. In addition, body temperature (preferably measured as oral temperature) as indicator of reactogenicity will be collected (with fever defined as body temperature ≥38.0°C/100.4°F).

For each solicited AE and fever, the percentage of subjects will be summarized by event severity for each day for the 7 days after each vaccine/placebo administration and overall. For subjects with more than 1 episode of the same event, the maximum severity will be used for tabulations.

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

In general, unsolicited AEs will be tabulated at each of the following levels: overall summary (subject with at least 1 AE), and by SOC and PT. Unsolicited AEs will be summarized as follows: by PT; by SOC and PT; by SOC, PT, and severity; by SOC, PT, and relationship (causality) to the investigational vaccine/placebo; and by SOC and PT, including events with frequency greater than a pre-defined frequency.

Safety summaries will be provided by dose group overall, as well as by baseline flavivirus serostatus.

13.2 Interim Analysis and Sequence of Analyses

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, including safety data from all flavivirus primed subjects up to Visit 4 (on Day 29, 28 days post dose 1).

The final 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 subsequent analyses will include (1) the data up to Visit 9 (12 months post dose 2) and (2) the data up to Visit 11 (24 months post dose 2). 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).

More details regarding the analyses will be provided in the SAP.

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, ie, Visit 11 (Day 757, 24 months post dose 2).

13.3 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.

14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Trial-Site Monitoring Visits

Monitoring visits to the trial site will be made periodically during the trial to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of data recorded on the eCRFs. Source documents are defined as original documents, data, and records. The investigator and institution guarantee access to source documents by the sponsor or its designee (CRO) and by the IRB or IEC.

All aspects of the trial and its documentation will be subject to review by the sponsor or designee (as long as blinding is not jeopardized), including but not limited to the Investigator's Binder, investigational vaccine/placebo, subject medical records, ICF documentation, documentation of subject authorization to use personal health information (if separate from the ICF), and review of eCRFs and associated source documents. It is important that the investigator and other trial personnel are available during the monitoring visits and that sufficient time is devoted to the process.

14.2 Protocol Deviations

The investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to trial subjects. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the investigator should consult with the medical monitor (and IRB or IEC, as required) to determine the appropriate course of action. There will be no exemptions (a prospective approved deviation) from the inclusion or exclusion criteria.

14.3 Quality Assurance Audits and Regulatory Agency Inspections

The trial site also may be subject to quality assurance audits by the sponsor or designees. In this circumstance, the sponsor-designated auditor will contact the site in advance to arrange an auditing visit. The auditor may ask to visit the facilities where laboratory samples are collected, where the vaccine/placebo is stored and prepared, and any other facility used during the trial. In addition, there is the possibility that this trial may be inspected by regulatory agencies, including those of foreign governments (eg, the FDA, the United Kingdom Medicines and Healthcare products Regulatory Agency, the Pharmaceuticals and Medical Devices Agency of Japan). If the trial site is contacted for an inspection by a regulatory body, the sponsor should be notified immediately. The investigator and institution guarantee access for quality assurance auditors to all trial documents as described in Section 14.1.

15.0 ETHICAL ASPECTS OF THE TRIAL

This trial will be conducted with the highest respect for the individual participants (ie, subjects) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki (1), and ICH E6 (2). Each investigator will conduct the trial according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the "Responsibilities of the Investigator" that are listed in Appendix A.

. The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

15.1 IRB and/or IEC Approval

IRBs and IECs must be constituted according to the applicable requirements of each participating region. The sponsor or designee will require documentation noting all names and titles of members who make up the respective IRB or IEC. If any member of the IRB or IEC has direct participation in this trial, written notification regarding his or her abstinence from voting must also be obtained.

The sponsor or designee will supply relevant documents for submission to the respective IRB or IEC for the protocol's review and approval. This protocol, the IB, a copy of the ICF, and, if applicable, subject recruitment materials and/or advertisements and other documents required by all applicable laws and regulations, must be submitted to a central or local IRB or IEC for approval. The IRB's or IEC's written approval of the protocol and subject ICF must be obtained and submitted to the sponsor or designee before commencement of the trial (ie, before shipment of the sponsor-supplied Vaccine or trial specific screening activity). The IRB or IEC approval must refer to the trial by exact protocol title, number, and version date; identify versions of other documents (eg, ICF) reviewed; and state the approval date. The sponsor will notify the site once the sponsor has confirmed the adequacy of site regulatory documentation and, when applicable, the sponsor has received permission from the competent authority to begin the trial. Until the site receives notification, no protocol activities, including screening may occur.

Sites must adhere to all requirements stipulated by their respective IRB or IEC. This may include notification to the IRB or IEC regarding protocol amendments, updates to the ICF, recruitment materials intended for viewing by subjects, local safety reporting requirements, reports and updates regarding the ongoing review of the trial at intervals specified by the respective IRB or IEC, and submission of the investigator's final status report to IRB or IEC. All IRB and IEC approvals and relevant documentation for these items must be provided to the sponsor or its designee.

Subject incentives should not exert undue influence for participation. Payments to subjects must be approved by the IRB or IEC and sponsor.

15.2 Subject Information, Informed Consent and Subject Authorization

Written consent documents will embody the elements of informed consent as described in the Declaration of Helsinki (1) and ICH E6 (2) and will be in accordance with all applicable laws and regulations. The ICF, subject authorization form (if applicable), and subject information sheet (if

applicable) describe the planned and permitted uses, transfers, and disclosures of the subject's personal and personal health information for purposes of conducting the trial. The ICF and the subject information sheet (if applicable) further explain the nature of the trial, its objectives, and potential risks and benefits, as well as the date informed consent / is given. The ICF will detail the requirements of the participant and the fact that he or she is free to withdraw at any time without giving a reason and without prejudice to his or her further medical care.

The investigator is responsible for the preparation, content, and IRB or IEC approval of the ICF and if applicable, the subject authorization form. The ICF, subject authorization form (if applicable), and subject information sheet (if applicable) must be approved by both the IRB or IEC and the sponsor prior to use.

The ICF, subject authorization form (if applicable), and subject information sheet (if applicable) must be written in a language fully comprehensible to the prospective subject. It is the responsibility of the investigator to explain the detailed elements of the ICF, subject authorization form (if applicable), and subject information sheet (if applicable) to the subject. Information should be given in both oral and written form whenever possible and in the manner deemed appropriate by the IRB or IEC.

The subject must be given ample opportunity to: (1) inquire about details of the trial and (2) decide whether or not to participate in the trial. If the subject determines he or she will participate in the trial, then the informed consent and subject authorization form (if applicable) must be signed and dated by the subject, at the time of consent and prior to the subject entering into the trial. The subject should be instructed to sign using their legal names, not nicknames, using blue or black ballpoint ink. The investigator must also sign and date the informed consent and subject authorization (if applicable) at the time of consent and prior to subject entering into the trial; however, the sponsor may allow a designee of the investigator to sign to the extent permitted by applicable law.

Once signed, the original ICF, subject authorization form (if applicable), and subject information sheet (if applicable) will be stored in the investigator's site file. The investigator must document the date the subject signs the ICF in the subject's medical record and eCRF. Copies of the signed ICF, the signed subject authorization form (if applicable), and subject information sheet (if applicable) shall be given to the subject.

All revised ICFs must be reviewed and signed by the relevant subject in the same manner as the original ICF. The date the revised consent was obtained should be recorded in the subject's medical record and eCRF, and the subject should receive a copy of the revised ICF.

15.3 Subject Confidentiality

The sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this trial, a subject's source data will only be linked to the sponsor's clinical trial database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes, such as sex, age, or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH E6 (2) and to verify compliance with this protocol, the sponsor requires the investigator to permit its monitor or designee's monitor, representatives from any regulatory authority (eg, FDA, Medicines and Healthcare products Regulatory Agency, Pharmaceuticals and Medical Devices Agency), the sponsor's designated auditors, and the appropriate IRBs and IECs to review the subject's original medical records (source data or documents), including, but not limited to, laboratory test result reports, electrocardiography reports, admission and discharge summaries for hospital admissions occurring during a subject's trial participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the ICF process (see Section 15.2).

Copies of any subject source documents that are provided to the sponsor must have certain personally identifiable information removed (ie, subject name, address, and other identifier fields not collected on the subject's eCRF).

15.4 Publication, Disclosure, and Clinical Trial Registration Policy

15.4.1 Publication and Disclosure

The results of this trial are expected to be published in a scientific journal. It is anticipated that clinical and laboratory co-investigators will participate in authorship. The order of authorship and choice of journal will be proposed by the sponsor to the principal investigator(s), to be eventually agreed upon by all authors. The data analysis center for this trial will provide the analyses needed for publication. Information regarding this trial will be posted on ClinicalTrials.gov.

15.4.2 Clinical Trial Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable law, regulation and guidance, the sponsor will, at a minimum register all clinical trials conducted in subjects that it sponsors anywhere in the world on ClinicalTrials.gov or other publicly accessible websites before trial initiation. The sponsor contact information, along with investigator's city, country, and recruiting status will be registered and available for public viewing.

15.4.3 Clinical Trial Results Disclosure

The sponsor will post the results of this clinical trial, regardless of outcome, on ClinicalTrials.gov or other publicly accessible websites, as required by applicable laws and/or regulations.

Trial completion corresponds to the date on which the final subject was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was terminated.

15.5 Insurance and Compensation for Injury

Each subject in the trial must be insured in accordance with the regulations applicable to the site where the subject is participating. If a local underwriter is required, then the sponsor or sponsor's designee will obtain clinical trial insurance against the risk of injury to clinical trial subjects. Refer

to the Clinical Study Site Agreement regarding the sponsor's policy on subject compensation and treatment for injury. If the investigator has questions regarding this policy, he or she should contact the sponsor or sponsor's designee.

16.0 REFERENCES

1. WMA. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. Jama. 2013;310(20):2191-4. Epub 2013/10/22.
2. ICH. Harmonised Tripartite Guideline E6(R1) Guideline for Good Clinical Practice. Step 4. June 1996. 1996 [27 January 2017]; Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf.
3. Zanluca C, Dos Santos CN. Zika virus - an overview. Microbes and infection. 2016;18(5):295-301. Epub 2016/03/20.
4. Musso D, Gubler DJ. Zika Virus. Clinical microbiology reviews. 2016;29(3):487-524. Epub 2016/04/01.
5. Carod-Artal FJ. Epidemiology and neurological complications of infection by the Zika virus: a new emerging neurotropic virus. Revista de neurologia. 2016;62(7):317-28. Epub 2016/03/19.
6. Mlakar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj M, Mraz J, et al. Zika Virus Associated with Microcephaly. The New England journal of medicine. 2016;374(10):951-8. Epub 2016/02/11.
7. Schuler-Faccini L, Ribeiro EM, Feitosa IM, Horovitz DD, Cavalcanti DP, Pessoa A, et al. Possible Association Between Zika Virus Infection and Microcephaly - Brazil, 2015. MMWR Morbidity and mortality weekly report. 2016;65(3):59-62. Epub 2016/01/29.
8. van der Linden V, Pessoa A, Dobyns W, Barkovich AJ, Junior HV, Filho EL, et al. Description of 13 Infants Born During October 2015-January 2016 With Congenital Zika Virus Infection Without Microcephaly at Birth - Brazil. MMWR Morbidity and mortality weekly report. 2016;65(47):1343-8. Epub 2016/12/03.
9. Moore CA, Staples JE, Dobyns WB, Pessoa A, Ventura CV, Fonseca EB, et al. Characterizing the Pattern of Anomalies in Congenital Zika Syndrome for Pediatric Clinicians. JAMA pediatrics. 2016. Epub 2016/11/05.
10. Reynolds MR, Jones AM, Petersen EE, Lee EH, Rice ME, Bingham A, et al. Vital Signs: Update on Zika Virus-Associated Birth Defects and Evaluation of All U.S. Infants with Congenital Zika Virus Exposure - U.S. Zika Pregnancy Registry, 2016. MMWR Morbidity and mortality weekly report. 2017;66(13):366-73. Epub 2017/04/07.
11. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, et al. Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014;19(9):7-9. Epub 2014/03/15.
12. Cao-Lormeau VM, Blake A, Mons S, Lastere S, Roche C, Vanhomwegen J, et al. Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet. 2016;387(10027):1531-9. Epub 2016/03/08.
13. PAHO. Cumulative Zika suspected and confirmed cases reported by countries and territories in the Americas, 2015-2016. Pan American Health Organization; 2016 [17 November 2016]; Available from: Cumulative Zika suspected and confirmed cases reported by countries and territories in the Americas, 2015-2016.

14. Carteaux G, Maquart M, Bedet A, Contou D, Brugieres P, Fourati S, et al. Zika Virus Associated with Meningoencephalitis. The New England journal of medicine. 2016;374(16):1595-6. Epub 2016/03/10.
15. Araujo LM, Ferreira ML, Nascimento OJ. Guillain-Barre syndrome associated with the Zika virus outbreak in Brazil. Arquivos de neuro-psiquiatria. 2016;74(3):253-5. Epub 2016/04/07.
16. Mecharles S, Herrmann C, Poullain P, Tran TH, Deschamps N, Mathon G, et al. Acute myelitis due to Zika virus infection. Lancet. 2016;387(10026):1481. Epub 2016/03/08.
17. WHO. Zika situation report. 8 December 2016. 2016 [14 December 2016]; Available from: http://www.who.int/emergencies/zika-virus/situation-report/8-december-2016/en/.
18. WHO. Zika - Epidemiological Update. World Health Organization; 2017 [updated 27 April 201705 May 2017]; Available from: http://www2.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=39706&lang=en.
19. Dos Santos T, Rodriguez A, Almiron M, Sanhueza A, Ramon P, de Oliveira WK, et al. Zika Virus and the Guillain-Barre Syndrome - Case Series from Seven Countries. The New England journal of medicine. 2016;375(16):1598-601. Epub 2016/11/01.
20. Dirlikov E, Major CG, Mayshack M, Medina N, Matos D, Ryff KR, et al. Guillain-Barre Syndrome During Ongoing Zika Virus Transmission - Puerto Rico, January 1-July 31, 2016. MMWR Morbidity and mortality weekly report. 2016;65(34):910-4. Epub 2016/09/02.
21. Krauer F, Riesen M, Reveiz L, Oladapo OT, Martinez-Vega R, Porgo TV, et al. Zika Virus Infection as a Cause of Congenital Brain Abnormalities and Guillain-Barre Syndrome: Systematic Review. PLoS medicine. 2017;14(1):e1002203. Epub 2017/01/04.
22. The L. Another kind of Zika public health emergency. Lancet. 2017;389(10069):573. Epub 2017/02/15.
23. Uncini A, Shahrizaila N, Kuwabara S. Zika virus infection and Guillain-Barre syndrome: a review focused on clinical and electrophysiological subtypes. Journal of neurology, neurosurgery, and psychiatry. 2017;88(3):266-71. Epub 2016/11/02.
24. Cauchemez S, Besnard M, Bompard P, Dub T, Guillemette-Artur P, Eyrolle-Guignot D, et al. Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study. Lancet. 2016;387(10033):2125-32. Epub 2016/03/20.
25. WHO. WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations. 2016 [30 November 2016]; Available from: http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-zika/en/.
26. Du Toit A. Zika virus structure, epidemiology and evolution. Nature Reviews Microbiology. 2016;14(267).
27. Sirohi D, Chen Z, Sun L, Klose T, Pierson TC, Rossmann MG, et al. The 3.8 A resolution cryo-EM structure of Zika virus. Science. 2016;352(6284):467-70. Epub 2016/04/02.
28. Pierson T, Diamond M. Flaviviruses. In: Knipe DM HP, editor. Fields Virology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. p. 747e94.

29. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1952;46(5):509-20. Epub 1952/09/01.
30. Macnamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1954;48(2):139-45. Epub 1954/03/01.
31. Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E, et al. Concurrent outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014;19(41):pii: 20929. Epub 2014/10/28.
32. Smithburn KC. Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa. J Immunol. 1952;69(2):223-34. Epub 1952/08/01.
33. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. The New England journal of medicine. 2009;360(24):2536-43. Epub 2009/06/12.
34. Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika virus, French polynesia, South pacific, 2013. Emerging infectious diseases. 2014;20(6):1085-6. Epub 2014/05/27.
35. Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fasce R, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Archives of virology. 2016;161(3):665-8. Epub 2015/11/28.
36. Zanluca C, Melo VC, Mosimann AL, Santos GI, Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Memorias do Instituto Oswaldo Cruz. 2015;110(4):569-72. Epub 2015/06/11.
37. WHO. Zika situation report - Zika virus, Microcephaly and Guillain-Barré syndrome. World Health Organization; 2017 [updated 10 March 201705 May 2017]; Available from: http://www.who.int/emergencies/zika-virus/situation-report/10-march-2017/en/.
38. Ikejezie J, Shapiro CN, Kim J, Chiu M, Almiron M, Ugarte C, et al. Zika Virus Transmission - Region of the Americas, May 15, 2015-December 15, 2016. MMWR Morbidity and mortality weekly report. 2017;66(12):329-34. Epub 2017/03/31.
39. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. eLife. 2015;4:e08347. Epub 2015/07/01.
40. Ayres CF. Identification of Zika virus vectors and implications for control. The Lancet Infectious diseases. 2016;16(3):278-9. Epub 2016/02/09.
41. Gubler DJ. The global emergence/resurgence of arboviral diseases as public health problems. Archives of medical research. 2002;33(4):330-42. Epub 2002/09/18.
42. Martines RB, Bhatnagar J, Keating MK, Silva-Flannery L, Muehlenbachs A, Gary J, et al. Notes from the Field: Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses--Brazil, 2015. MMWR Morbidity and mortality weekly report. 2016;65(6):159-60. Epub 2016/02/20.

43. Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, Fabri A, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. The Lancet Infectious diseases. 2016;16(6):653-60. Epub 2016/02/22.
44. Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2016;47(1):6-7. Epub 2016/01/06.
45. Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014;19(13):20751. Epub 2014/04/12.
46. Dupont-Rouzeyrol M, Biron A, O'Connor O, Huguon E, Descloux E. Infectious Zika viral particles in breastmilk. Lancet. 2016;387(10023):1051. Epub 2016/03/06.
47. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerging infectious diseases. 2011;17(5):880-2. Epub 2011/05/03.
48. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. Emerging infectious diseases. 2015;21(2):359-61. Epub 2015/01/28.
49. D'Ortenzio E, Matheron S, Yazdanpanah Y, de Lamballerie X, Hubert B, Piorkowski G, et al. Evidence of Sexual Transmission of Zika Virus. The New England journal of medicine. 2016;374(22):2195-8. Epub 2016/04/14.
50. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014;19(14). Epub 2014/04/18.
51. Vasquez AM, Sapiano MR, Basavaraju SV, Kuehnert MJ, Rivera-Garcia B. Survey of Blood Collection Centers and Implementation of Guidance for Prevention of Transfusion-Transmitted Zika Virus Infection--Puerto Rico, 2016. MMWR Morbidity and mortality weekly report. 2016;65(14):375-8. Epub 2016/04/15.
52. Chen LH, Wilson ME. Transmission of dengue virus without a mosquito vector: nosocomial mucocutaneous transmission and other routes of transmission. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;39(6):e56-60. Epub 2004/10/09.
53. CDC. Clinical Guidance for Healthcare Providers for Prevention of Sexual Transmission of Zika Virus. 2016 [01 December 2016]; Available from: http://www.cdc.gov/zika/hc-providers/clinical-guidance/sexualtransmission.html.
54. Landry ML, St George K. Laboratory Diagnosis of Zika Virus Infection. Archives of pathology & laboratory medicine. 2017;141(1):60-7. Epub 2016/10/21.
55. Parra B, Lizarazo J, Jimenez-Arango JA, Zea-Vera AF, Gonzalez-Manrique G, Vargas J, et al. Guillain-Barre Syndrome Associated with Zika Virus Infection in Colombia. The New England journal of medicine. 2016;375(16):1513-23. Epub 2016/11/01.

56. Murray KO, Gorchakov R, Carlson AR, Berry R, Lai L, Natrajan M, et al. Prolonged Detection of Zika Virus in Vaginal Secretions and Whole Blood. Emerging infectious diseases. 2017;23(1):99-101. Epub 2016/10/18.
57. Miner JJ, Sene A, Richner JM, Smith AM, Santeford A, Ban N, et al. Zika Virus Infection in Mice Causes Panuveitis with Shedding of Virus in Tears. Cell reports. 2016;16(12):3208-18. Epub 2016/09/11.
58. Swaminathan S, Schlaberg R, Lewis J, Hanson KE, Couturier MR. Fatal Zika Virus Infection with Secondary Nonsexual Transmission. The New England journal of medicine. 2016;375(19):1907-9. Epub 2016/09/30.
59. WHO. Zika Virus. Transmission and risks. World Health Organization; 2017 [updated 28 April 201705 May 2017]; Available from: https://www.cdc.gov/zika/transmission/index.html.
60. WHO. Zika virus. Fact sheet. 2017 [updated September 201605 May 2017]; Available from: http://www.who.int/mediacentre/factsheets/zika/en/.
61. ECDC. Rapid risk assessment: Zika virus disease epidemic. Tenth update. Stockholm: European Centre for Disease Prevention and Control; 2017 [updated 04 April 201705 May 2017]; Available from: http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1671#sthash.LjiTcDRg.dpuf.
62. CDC. Guidance for U.S. Laboratories Testing for Zika Virus Infection. November 16, 2016. 2016 [21 December 2016]; Available from: https://www.cdc.gov/zika/laboratories/lab-guidance.html.
63. WHO. Laboratory testing for Zika virus infection. Interim guidance. WHO/ZIKV/LAB/1612016.
64. CDC. Guidance for U.S. Laboratory Testing for Zika Virus Infection: Implications for Health Care Providers. Announcement November 25, 2016. 2016 [21 December 2016]; Available from: https://www.cdc.gov/mmwr/volumes/65/wr/mm6546a7.htm?s_cid=mm6546a7_w.
65. Barzon L, Pacenti M, Berto A, Sinigaglia A, Franchin E, Lavezzo E, et al. Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Dominican Republic to Italy, January 2016. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2016;21(10). Epub 2016/03/19.
66. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2015;68:53-5. Epub 2015/06/14.
67. Driggers RW, Ho CY, Korhonen EM, Kuivanen S, Jaaskelainen AJ, Smura T, et al. Zika Virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities. The New England journal of medicine. 2016;374(22):2142-51. Epub 2016/03/31.
68. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerging infectious diseases. 2008;14(8):1232-9. Epub 2008/08/06.

69. Dowd KA, Ko SY, Morabito KM, Yang ES, Pelc RS, DeMaso CR, et al. Rapid development of a DNA vaccine for Zika virus. Science. 2016;354(6309):237-40. Epub 2016/10/07.
70. Zammarchi L, Stella G, Mantella A, Bartolozzi D, Tappe D, Gunther S, et al. Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2015;63:32-5. Epub 2015/01/21.
71. Paul LM, Carlin ER, Jenkins MM, Tan AL, Barcellona CM, Nicholson CO, et al. Dengue virus antibodies enhance Zika virus infection. Clinical & translational immunology. 2016;5(12):e117. Epub 2017/01/17.
72. Brasil P, Pereira JP, Jr., Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. Zika Virus Infection in Pregnant Women in Rio de Janeiro. The New England journal of medicine. 2016;375(24):2321-34. Epub 2016/03/05.
73. CDC. Zika Virus Treatment. 2016 [23 December 2016]; Available from: https://www.cdc.gov/zika/symptoms/treatment.html.
74. CDC. Developmental disabilities. Atlanta, GA: US Department of Health and Human Services2015 [21 December 2016]; Available from: http://www.cdc.gov/ncbddd/developmentaldisabilities/index.html.
75. Meena AK, Khadilkar SV, Murthy JM. Treatment guidelines for Guillain-Barre Syndrome. Annals of Indian Academy of Neurology. 2011;14(Suppl 1):S73-81. Epub 2011/08/19.
76. CDC. CDC's Response to Zika. Updated Interim Pregnancy Guidance. 2016 [01 February 2017]; Available from: https://www.cdc.gov/zika/pdfs/testing_algorithm.pdf.
77. Nasci RS, Wirtz RA, Brogdon WG. Protection against mosquitoes, ticks, and other arthropods. In: CDC health information for international travel. New York, NY: Oxford University Press; 2015 [21 December 2016]; Available from: http://wwwnc.cdc.gov/travel/yellowbook/2016/the-pre-travel-consultation/protection-against-mosquitoes-ticks-other-arthropods.
78. CDC. Travel notices. Atlanta, GA: US Department of Health and Human Services2016 [21 December 2016]; Available from: http://wwwnc.cdc.gov/travel/notices.
79. HHS. HHS declares a public health emergency in Puerto Rico in response to Zika outbreak. U.S. Department of Health & Human Services (HHS) Press Office2016 [30 November 2016]; Available from: https://www.hhs.gov/about/news/2016/08/12/hhs-declares-public-health-emergency-in-puerto-rico-in-response-to-zika-outbreak.html.
80. WHO. WHO statement. Fifth meeting of the Emergency Committee under the International Health Regulations (2005) regarding microcephaly, other neurological disorders and Zika virus. 18 November 2016. 2016 [02 December 2016]; Available from: http://www.who.int/mediacentre/news/statements/2016/zika-fifth-ec/en/.
81. Yang YT, Sarfaty M. Zika virus: A call to action for physicians in the era of climate change. Preventive medicine reports. 2016;4:444-6. Epub 2016/09/13.
82. HHS. HHS awards $ 19.8 million to Japanese company to accelerate development of a Zika vaccine. U.S. Department of Health & Human Services (HHS) Press Office2016 [14

December 2016]; Press release 1 September 2016]. Available from: https://www.hhs.gov/about/news/2016/09/01/hhs-awards-19-8-million-japanese-company-accelerate-development-zika-vaccine.html.

83. Dowd KA, DeMaso CR, Pelc RS, Speer SD, Smith AR, Goo L, et al. Broadly Neutralizing Activity of Zika Virus-Immune Sera Identifies a Single Viral Serotype. Cell reports. 2016;16(6):1485-91. Epub 2016/08/03.
84. WRAIR, NIAID. A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule With a Zika Virus Purified Inactivated Vaccine Plus Alum Adjuvant in Healthy Adults. ClinicalTrials.gov Identifier: NCT02937233; 2016 [16 January 2017]; Available from: https://clinicaltrials.gov/ct2/show/NCT02937233?term=zika+wrair&rank=1.
85. Abbink P, Larocca RA, De La Barrera RA, Bricault CA, Moseley ET, Boyd M, et al. Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. Science. 2016;353(6304):1129-32. Epub 2016/08/06.
86. ICH. Harmonised Tripartite Guideline E8 General Considerations for Clinical Trials. Step 4. July 1997. 1997 [10 January 2017]; Available from: http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/general-considerations-for-clinical-trials.html.
87. WHO. Clinical evaluation. Guidelines on clinical evaluation of vaccines: regulatory expectations. Fifty-second report, Geneva, ECBS, 2004;TRS 924,Annex 1. 2004 [10 January 2017]; Available from: http://www.who.int/biologicals/publications/trs/areas/vaccines/clinical_evaluation/en/.
88. ICH. Harmonised Tripartite Guideline E4 Dose-Response Information to Support Drug Registration. Step 4. March 1994. 1994 [10 January 2017]; Available from: http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/dose-response-information-to-support-drug-registration.html.
89. FDA. Guidance for Industry. Exposure-Response Relationships — Study Design, Data Analysis, and Regulatory Applications. 2003 [10 January 2017]; Available from: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm072109.pdf.
90. ICH. Harmonised Tripartite Guideline E10 Choice of Control Group and Related Issues in Clinical Trials. Step 4. July 2000. 2000 [10 January 2017]; Available from: http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/choice-of-control-group-and-related-issues-in-clinical-trials.html.
91. WHO. Weekly epidemiological record. Reducing pain at the time of vaccination: September 2015. WHO position paper. 2015;90(39):505-16.
92. Kohl KS, Marcy SM, Blum M, Connell Jones M, Dagan R, Hansen J, et al. Fever after immunization: current concepts and improved future scientific understanding. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;39(3):389-94. Epub 2004/08/13.

93. 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 Responsibilities of the Investigator

Clinical research studies sponsored by the sponsor are subject to ICH GCP and all the applicable local laws and regulations.

The investigator agrees to assume the following responsibilities:

1. Conduct the trial in accordance with the protocol.
2. Personally conduct or supervise the staff who will assist in the protocol.
3. Ensure that trial related procedures, including trial specific (non-routine/non-standard panel) screening assessments are NOT performed on potential subjects, prior to the receipt of written approval from relevant governing bodies/authorities.
4. Ensure that all colleagues and employees assisting in the conduct of the trial are informed of these obligations.
5. Secure prior approval of the trial and any changes by an appropriate IRB/IEC that conform to 21 CFR Part 56, ICH, and local regulatory requirements.
6. Ensure that the IRB/IEC will be responsible for initial review, continuing review, and approval of the protocol. Promptly report to the IRB/IEC all changes in research activity and all anticipated risks to subjects. Make at least yearly reports on the progress of the trial to the IRB/IEC, and issue a final report within 3 months of trial completion.
7. Ensure that requirements for informed consent, as outlined in 21 CFR Part 50, ICH, and local regulations, are met.
8. Obtain valid informed consent from each subject who participates in the trial, and document the date of consent in the subject's medical chart. Valid ICF is the most current version approved by the IRB/IEC. Each ICF should contain a subject authorization section that describes the uses and disclosures of a subject's personal information (including personal health information) that will take place in connection with the trial. If an ICF does not include such a subject authorization, then the investigator must obtain a separate subject authorization form from each subject.
9. Prepare and maintain adequate case histories of all persons entered into the trial, including eCRFs, hospital records, laboratory results, etc., and maintain these data for a minimum of 2 years following notification by the sponsor that all investigations have been discontinued or that the regulatory authority has approved the marketing application. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.
10. Allow possible inspection and copying by the regulatory authority of GCP-specified essential documents.
11. Maintain current records of the receipt, administration, and disposition of sponsor-supplied vaccines, and return all unused sponsor-supplied vaccines to the sponsor.
12. Report AEs to the sponsor promptly. In the event of an SAE, notify the sponsor within 24 hours.
13. Review and provide a signature as approval of the content of the clinical study report.

Appendix B Elements of the Subject Informed Consent Form

In seeking informed consent, the following information shall be provided to each subject:

1. A statement that the trial involves research.
2. An explanation of the purposes of the research.
3. The expected duration of the subject's participation.
4. A description of the procedures to be followed, including invasive procedures.
5. The identification of any procedures that are experimental.
6. The estimated number of subjects involved in the trial.
7. A description of the subject's responsibilities.
8. A description of the conduct of the trial.
9. A statement describing the vaccination(s) and the probability for random assignment to each treatment.
10. A description of the possible side effects following vaccine administration that the subject may receive.
11. A description of any reasonably foreseeable risks or discomforts to the subject and, when applicable, to an embryo, fetus, or nursing infant.
12. A description of any benefits to the subject or to others that reasonably may be expected from the research. When there is no intended clinical benefit to the subject, the subject should be made aware of this.
13. Disclosures of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject and their important potential risks and benefits.
14. A statement describing the extent to which confidentiality of records identifying the subject will be maintained, and a note of the possibility that regulatory agencies, auditor(s), IRB/IEC, and the monitor may inspect the records. By signing a written informed consent, the subject is authorizing such access.
15. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of or where further information may be obtained.
16. The anticipated prorated payment(s), if any, to the subject for participating in the trial.
17. The anticipated expenses, if any, to the subject for participating in the trial.
18. An explanation of whom to contact for answers to pertinent questions about the research (investigator), subject's rights, and IRB/IEC and whom to contact in the event of a research-related injury to the subject.
19. A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject otherwise is entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.
20. The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.
21. A statement that the subject will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the trial.

22. A statement that results of pharmacogenomic analysis will not be disclosed to an individual, unless prevailing laws require the sponsor to do so.
23. The foreseeable circumstances or reasons under which the subject's participation in the trial may be terminated.
24. A written subject authorization (either contained within the informed consent or provided as a separate document) describing to the subject the contemplated and permissible uses and disclosures of the subject's personal information (including personal health information) for purposes of conducting the trial. The subject authorization must contain the following statements regarding the uses and disclosures of the subject's personal information:
    a) that personal information (including personal health information) may be processed by or transferred to other parties in other countries for clinical research and safety reporting purposes, including, without limitation, to the following: (1) Takeda, its affiliates, and licensing partners; (2) business partners assisting Takeda, its affiliates, and licensing partners; (3) regulatory agencies and other health authorities; and (4) IRBs/IECs;
    b) it is possible that personal information (including personal health information) may be processed and transferred to countries that do not have data protection laws that offer subjects the same level of protection as the data protection laws within this country; however, Takeda will make every effort to keep your personal information confidential, and your name will not be disclosed outside the clinic unless required by law;
    c) that personal information (including personal health information) may be added to Takeda's research databases for purposes of developing a better understanding of the safety and effectiveness of the investigational vaccine(s), studying other therapies for patients, developing a better understanding of disease, and improving the efficiency of future clinical studies;
    d) that subjects agree not to restrict the use and disclosure of their personal information (including personal health information) upon withdrawal from the trial to the extent that the restricted use or disclosure of such information may impact the scientific integrity of the research; and
    e) that the subject's identity will remain confidential in the event that trial results are published.
25. The vaccination may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) that are currently unforeseeable. Female subjects of childbearing potential, sexually active, must use "acceptable contraceptive methods" for at least 2 months prior to trial entry through to 2 months after the last dose of investigational vaccine/placebo. Regular pregnancy tests will be performed throughout the trial for all female subjects of childbearing potential. If a subject is found to be pregnant during trial, no further vaccine doses will be administered and the investigator will offer the subject the choice to receive unblinded treatment information. In addition, female subject of childbearing potential must be advised not to donate ova during the study period.
26. To avoid sexual transmission of ZIKV from natural exposure: Sexually active subjects (both male and female) should use latex condoms correctly and consistently even if other contraceptive measures are used from signing the ICF through the end of the trial. Male subjects must be advised not to donate sperm during this period.

27. A statement that clinical trial information from this trial will be publicly disclosed in a publicly accessible website, such as ClinicalTrials.gov.
28. Subject intending to donate blood/blood product for transfusion might have positive ZIKV serological test result due to the participation in this study. Subject will be required to inform the health professional about the participation in the ZIKV vaccine study when intending to donate blood/blood product.

Appendix C Investigator Consent to Use of Personal Information

Takeda will collect and retain personal information of investigator, including his or her name, address, and other personally identifiable information. In addition, investigator's personal information may be transferred to other parties located in countries throughout the world (eg, the United Kingdom, United States, and Japan), including the following:

- Takeda, its affiliates, and licensing partners.
- Business partners assisting Takeda, its affiliates, and licensing partners.
- Regulatory agencies and other health authorities.
- IRBs and IECs.

Investigator's personal information may be retained, processed, and transferred by Takeda and these other parties for research purposes including the following:

- Assessment of the suitability of investigator for the trial and/or other clinical studies.
- Management, monitoring, inspection, and audit of the trial.
- Analysis, review, and verification of the trial results.
- Safety reporting and pharmacovigilance relating to the trial.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to the trial.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to other vaccines used in other clinical studies that may contain the same chemical compound present in the investigational vaccine.
- Inspections and investigations by regulatory authorities relating to the trial.
- Self-inspection and internal audit within Takeda, its affiliates, and licensing partners.
- Archiving and audit of trial records.
- Posting investigator site contact information, trial details and results on publicly accessible clinical trial registries, databases, and websites.

Investigator's personal information may be transferred to other countries that do not have data protection laws that offer the same level of protection as data protection laws in investigator's own country. Investigator acknowledges and consents to the use of his or her personal information by Takeda and other parties for the purposes described above.

Appendix D 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

Appendix E 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 (93)

Table 16.a 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 16.b 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)]	ï€ 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 16.c 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 16.d 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.

Signature Page for ZIK-101 Protocol Amendment 3, Version 7.0, 12 September 2018
Title: A Phase 1, Randomized, Observer-Blind, Placebo-Controlled, Safety, Immuno

Approval	PPD
Approval	
Approval	
Approval	

Document Number: TAK-426-00159 v6.0