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TITLE:

A Phase I Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Immunogenicity of a Live-Attenuated Tetravalent Dengue Vaccine (V181) in Flavivirus-Naïve and Flavivirus-Experienced Healthy Adults

IND NUMBER: To Be Determined
EudraCT NUMBER: Not Applicable

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SUMMARY OF CHANGES

PRIMARY REASON FOR THIS AMENDMENT:

Section Number	Section Title	Description of Change	Rationale
2.2; 3.3; 4.2.1; 4.2.3.1; 4.2.3.2; 5.1.2; 5.1.3.2; 6.0; 7.1.2.4; 7.1.2.5; 7.1.3.1; 7.1.3.4; 8.1; 8.4.1; 8.6.2; 8.7; 12.3	Trial Diagram, Figure 1 and Figure 2; Exploratory Objectives; Rationale for the Trial and Selected Subject Population; Safety Endpoints; Rationale for Immunogenicity Measurements and Assay Methods; Subject Inclusion Criteria; Criteria for Excluding Subjects from Receiving Vaccination 2: Trial Flow Chart; Assessment of Adverse Events (Review of Vaccination Report Card); Assessment of Rash; Laboratory Safety Evaluations (Hematology, Chemistry, and Urinalysis); Viremia RT-PCR Testing; Statistical Analysis Plan Summary; Immunogenicity Endpoints; Statistical Methods for Safety Analysis; Interim Analysis; Approximate Blood/Tissue Volumes Drawn/Collected by Trial Visit and Sample Type	An additional trial visit (Visit 5) was added at 56 days Postdose 1 (Sections 2.2; 4.2.3.2; 6.0; and 12.3). At Visit 5, a sample for immunogenicity testing will be obtained (Sections 6.0 and 12.3). Adverse events, new medical history, and concomitant medication data will be collected (Section 6.0).	The purpose of the additional trial visit at 56 days Postdose 1 is primarily to obtain additional immunogenicity data following the first dose of vaccine at this time point. The 28 days Postdose 1 time point will remain the primary immunogenicity time point in the trial.

ADDITIONAL CHANGES FOR THIS AMENDMENT:

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
2.1	Trial Design	Text was added to clarify that Leibovitz L-15 is to be used **as the diluent for field mix formulation**, in addition to being used as placebo.	Leibovitz L-15 medium will be utilized for both placebo and as diluent for field mix formulation.
2.1; 4.2.3.1; 6.0; 7.1.3.4; 7.1.3.5.1.1; 12.3	Trial Design; Safety Endpoints; Trial Flow Chart; Viremia RT-PCR Testing; RT-PCR for Dengue	The language was updated to clarify the type of dengue RT-PCR assays (qualitative and/or quantitative): Virologically-confirmed dengue is defined as 2 or more consecutive days of fever ≥38.0°C (≥100.4°F) with a positive qualitative ~~**or**~~ **and/or** quantitative serotype-specific RT-PCR assay.	A dengue RT-PCR assay is in development and the description was clarified that 1 or both types of results (qualitative and/or quantitative) will be available for the study.
2.2	Trial Diagram, Figure 1 and Figure 2	Treatment group TV003 was changed to TV005 to reflect the correct active treatment groups.	Treatment group TV003 was inadvertently listed twice and treatment group TV005 was not listed at all; therefore, the figures were updated to align with the trial design.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
2.2	Trial Diagram, Figure 1 and Figure 2	Both Visit 2 and Visit 6 were updated to include "postvaccination" in the brief description of visit procedures. Specific day ranges for the visits (e.g., Visit 2 - Day 7, 7 to 9 days Postdose 1) were replaced by the permitted visit window displayed in the study flow chart (e.g., Visit 2: 7 days Postdose 1, +2 days).	Clarifications were made to the trial diagrams to avoid confusion by study site personnel, and so that the diagrams are consistent with descriptions in the study flow chart.
3.2, 3.3; 4.2.3.2; 6.0; 7.1.3.3.1; 7.1.3.3.2; 7.1.3.5.2.1; 8.1; 8.4.1; 8.6.1; 12.3	Secondary Objective(s) and Hypothesis(es); Exploratory Objectives; Rationale for Immunogenicity Measurements and Assay Methods; Trial Flow Chart; Dengue IgG ELISA; Virus Reduction Neutralization (VRNT) for DENV1, DENV2, DENV3, and DENV4; VRNT for DENV1, DENV2, DENV3, and DENV4; Statistical Analysis Plan Summary; Immunogenicity Endpoints; Statistical Methods for Immunogenicity Analyses; Approximate Blood/Tissue Volumes Drawn/Collected by Trial Visit and by Sample Types	The secondary and exploratory objectives were updated to reflect a change to the assay name for the immunogenicity endpoints: Virus Reduction Neutralization Test at 50% neutralization ($VRNT_{50}$) was changed to **Virus Reduction Neutralization Test (VRNT)**.	The VRNT assay description was made more generic, because assay development is ongoing and the percent neutralization of virus activity to be used in the assay will not be available before the protocol amendment is finalized. The change in assay description was to delete the statement that the read out of the assay will be the titer that results in 50% virus neutralization. The secondary and exploratory objectives otherwise remain the same.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
4.2.3.1; 6.0; 7.1.5.3; 8.1; 8.4.1; 8.7; 8.9.1	Safety Endpoints; Trial Flow Chart; Discontinued Subjects Continuing to be Monitored in the Trial; Statistical Analysis Plan Summary; Immunogenicity Endpoints; Interim Analyses; Immunogenicity Analyses	The descriptions of the time points in the trial were edited; e.g., Day 28 Postdose 1 was changed to 28 days Postdose 1.	The terminology for visit time points was updated to clarify by how many days following each dose to schedule a visit.
4.2.3.1	Safety Endpoints	The following sentence was updated to clarify the Day 28 visit: During this period, subject visits will occur on 7, 12, and **28** days following each vaccination for safety follow-up, including safety laboratory measurements and qualitative and/or quantitative RT-PCR for the detection of vaccine virus viremia.	The visit at 28 days following vaccination was specified in order to display the 3 time points for which safety laboratory testing will be conducted.
4.2.3.1	Safety Endpoints	For subjects with suspected dengue, the type of specimen collected for the dengue RT-PCR test at the acute initial evaluation visit was updated to indicate that it should be **serum**.	Specimen type was clarified.
4.2.3.2	Rationale for Immunogenicity Measurements and Assay Methods	The term 'peak' was removed: The time point of 28 days was chosen based on the ~~peak~~ immunogenicity results seen in the NIH clinical trials to date.	The time point of 28 days Postdose 1 was selected for the primary endpoint based on overall NIH clinical trial immunogenicity results to date, and not specifically on peak immunogenicity results.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
4.2.3.3	Planned Exploratory Biomarker Research	The Planned Genetic Analysis was removed.	The Planned Genetic Analysis was inadvertently retained in the protocol, but is not relevant to this vaccine trial.
5.1.3	Subject Exclusion Criteria	Removed exclusion criterion 5: Subject has body mass index (BMI) >32kg/m^2.	Initially, the upper limit to BMI was intended as a surrogate for good health. Upon further consideration, it was determined that BMI is not a good measurement to evaluate health, and it is not standard practice to use BMI to assess subject health in vaccine clinical trials.
5.1.3.2	Criteria for Excluding Subjects from Receiving Vaccination 2	The word 'exclusion' was deleted: These subject ~~exclusion~~ criteria must be reviewed for each subject prior to vaccination 2 to ensure that none of the criteria apply to the subject.	The word 'exclusion' was deleted, because these criteria refer to both inclusion and exclusion criteria.
5.1.3.2	Criteria for Excluding Subjects from Receiving Vaccination 2	Exclusion criterion 7 was added: **Has performed unusual, unaccustomed strenuous, vigorous physical exercise/activity (e.g., beginning new weight-lifting, running, or bicycling regimens) within 72 hours prior to vaccination 2, or does not agree to refrain from unusual, unaccustomed strenuous, vigorous physical exercise/activity through 28 days following vaccination 2.**	The addition of exclusion criterion 7 is consistent with the protocol procedures described in Section 7.1.1.8.2.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
5.2.3	Trial Blinding	The following clarification was added: **Laboratory personnel will remain blinded to treatment group throughout the duration of the study.**	Trial blinding includes the blinding of laboratory personnel to treatment group throughout the duration of the study.
5.2.3; 8.2	Trial Blinding; Responsibility for Analyses/In-House Blinding	The following clarification was added: After the IA is released, Merck Headquarters (HQ) personnel **involved in the conduct of the trial** will be unblinded.	The text was clarified to specify the Merck HQ personnel who will be unblinded after the IA is released
5.5	Concomitant Medications/Vaccinations (Allowed & Prohibited)	Minor clarifications of receipt of live vaccine and enrollment in another clinical trial as prohibited were added.	The clarifications were added for consistency in the section.
6.0	Trial Flow Chart	Added "**Distribute Dengue Informational Brochure**" to screening procedures.	Distribution of the Dengue Informational Brochure was added as this was previously missing in the Trial Flow Chart.
6.0	Trial Flow Chart	The urine pregnancy screening procedure was removed from Footnote d.	The urine pregnancy test is not performed at the screening visit.
6.0	Trial Flow Chart	Time Relative to Dose 1 row was removed from Trial Flow Chart with Study Procedures for All Subjects (Day 1 to Year 1 PD2).	The time relative to Dose 1 was removed because the flow chart has been updated to capture time relative to Dose 1 separately from time relative to Dose 2.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
6.0	Trial Flow Chart	Sample blood volumes were removed from the trial flow chart.	Study site personnel are to reference the laboratory manual, which will provide a comprehensive resource for sample blood volumes.
6.0	Trial Flow Chart	In the screening procedures flow chart, footnote c was updated to clarify that medical history would be collected for 5 years prior to screening.	The update provides clarification for study procedures.
6.0	Trial Flow Chart	The following steps for using the Interactive Response Technology (IRT) were added to the trial flow charts: • **Screening procedures - Enter subject in IRT** • **Study procedures - Initiate Dose 2 in IRT**	These updates provide clarification for study procedures.
7.1.1.9; 7.1.2.4; 7.1.2.5; 7.1.5.3; 12.5; 12.6	Dispense Electronic Vaccination Report Cards; Assessment of Adverse Events (Review of Vaccination Report Card); Assessment of Rash; Discontinued Subjects Continuing to be Monitored in the Trial; List of Abbreviations; Adverse Events Toxicity Grading Scale	Vaccination Report Card (VRC) was changed to electronic Vaccination Report Card (eVRC).	The collection of VRC data was clarified to specify that VRC data will be captured electronically. A paper-based VRC will not be used in this trial.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
7.1.2.4	Assessment of Adverse Events (Review of eVRC)	Removed Table 1 reference in Appendix 12.6.	The reference to Table 1 was removed because there is no Table 1 in Appendix 12.6.
7.1.2.6	Assessment of Suspected Dengue Disease	Inserted text: **For the first 28 days after each vaccination, subjects should measure and record their temperature daily on the eVRC. Outside of those 28-day periods following each vaccination, subjects should take and record their temperature only when they feel febrile until the febrile episode resolves.**	This language was included in the Dengue Informational Brochure for subjects, but was inadvertently omitted from the protocol and was added to align both study documents.
7.1.2.6; 7.1.3.5.2	Assessment of Suspected Dengue Disease; Convalescent Visit	The words "to occur" were added to the following sentence: If the investigator assesses a case as possible/probable dengue, a subsequent convalescent visit will be scheduled **to occur** 14 to 28 days after the initial evaluation visit to assess if the subject has recovered from the suspected dengue case.	Clarification added to reflect the timing for scheduling the convalescent visit.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
7.1.3.2	Pregnancy Testing	The location where the serum-β-hCG pregnancy test is performed was changed from the local laboratory to the central laboratory. In addition, the location where the urine β-hCG pregnancy test is performed was changed from the local laboratory to the site.	The text was clarified to show that all serum-β-hCG pregnancy testing will be performed at the central laboratory, and all urine pregnancy testing will be conducted at the site.
7.1.3.4	Viremia RT-PCR Testing	The viremia RT-PCR testing at 7 and 28 days following each vaccination was added.	The viremia RT-PCR time points for 7 and 28 days following each vaccination were inadvertently omitted in this section.
7.1.3.7	Future Biomedical Research	The following bullet point was added: **Leftover main study serum from assay development stored for future research.**	The bullet point was added for consistency with information in footnote "i" in the study flow chart: Leftover main study serum will be stored for future biomedical research if the subject consents to future biomedical research.
9.1	Investigational Product, Table 7	The titer description was changed from >4.0E+03 pfu/mL to ≥4.0E+03 pfu/mL.	A correction was made to the titer cutoff, which is ≥4.0E+03 pfu/mL.

Section Number (s)	Section Title (s)	Description of Change (s)	Rationale
1.0; 2.1; 3.3; 4.1.1.5.1; 4.2.3.1; 5.1.3, 5.1.3.2; 5.2; 6.0; 7.1.2.4; 7.1.2.6; 7.1.3.3.1; 7.1.3.3.2; 7.1.3.5.1.2; 7.3.1; 8.1; 8.4.1; 8.6.1; 8.10; 9.1; 9.2; 12.3	Trial Summary; Trial Design; Exploratory Objects; Merck Live-Attenuated Tetravalent Vaccine (V181); Safety Endpoints; Subject Exclusion Criteria; Criteria for Excluding Subjects from Receiving Vaccination 2; Vaccination(s); Trial Flow Chart; Assessment of Adverse Events (Review of Vaccination Report Card); Assessment of Suspected Dengue Disease; Dengue IgG ELISA; Virus Reduction Neutralization Test for DENV1, DENV2, DENV3, and DENV4; RT-PCR for Chikungunya and Zika; Packaging and Labeling Information; Statistical Analysis Plan Summary; Immunogenicity Endpoints; Statistical Methods for Immunogenicity Analyses; Data Monitoring Committee; Subgroup Analyses; Investigational Product; Approximate Blood/Tissue Volumes Drawn/Collected by Trial Visit and by Sample Types	Minor edits were made as needed throughout the protocol to correct or clarify grammar and/or typographical errors.	Minor grammar and typographical edits were made to improve the clarity and readability of the protocol amendment.

1.0 TRIAL SUMMARY

Abbreviated Title	Phase I Live-Attenuated Tetravalent Vaccine (LATV) Dengue Vaccine Study
Sponsor Product Identifiers	V181
Trial Phase	Phase I
Clinical Indication	Active immunization to protect against disease caused by any of the 4 dengue viruses (DENV; DENV1, DENV2, DENV3, and DENV4) from age 1 to 70 years.
Trial Type	Interventional
Type of control	Placebo
Route of administration	Subcutaneous injection
Trial Blinding	Double-blind
Vaccination Groups	TV003 [rDENVlΔ30 (a dengue 1 vaccine); rDENV2/4Δ30(ME) (a dengue 2 vaccine); rDENV3Δ30/31 (a dengue 3 vaccine); and rDENV4Δ30 (a dengue 4 vaccine); similar concentrations for each component] TV005 [rDENVlΔ30 (a dengue 1 vaccine); rDENV2/4Δ30(ME) (a dengue 2 vaccine); rDENV3Δ30/31 (a dengue 3 vaccine); and rDENV4Δ30 (a dengue 4 vaccine); similar concentrations for all components except rDENV2/4Δ30(ME) which has 10-fold higher concentration] Placebo (Leibovitz L-15 medium)
Number of trial subjects	Approximately 200 subjects will be enrolled.
Estimated duration of trial	The Sponsor estimates that the trial will require approximately 1.5 years from the time the first subject signs the informed consent until the last subject's last study-related phone call or visit. For purposes of analysis and reporting, the overall trial ends when the Sponsor receives the last external clinical data point, e.g., serology assay result, or subject data from the last study-related contact (e.g., phone call, text, email, or visit).
Duration of Participation	Each subject will participate in the trial for approximately 1.5 years from the time the subject signs the Informed Consent Form (ICF) through the final study visit. Each subject will receive assigned treatment at Day 1 and Month 6. After the second vaccination each subject will be followed for 1 year.

Randomization Ratio	2:2:1

A list of abbreviations used in this document can be found in Section 12.5.

2.0 TRIAL DESIGN

2.1 Trial Design

This is a 3-arm, randomized, placebo-controlled, multi-center (continental United States and Puerto Rico), blinded trial of a live-attenuated tetravalent dengue vaccine (V181) in flavivirus-naïve and flavivirus-experienced 18- to 50-year-old healthy adults to be conducted in conformance with Good Clinical Practices (GCP). Subjects will be stratified by geography (continental United States and Puerto Rico) using an interactive response technology (IRT). The study will assess the safety, tolerability, and immunogenicity of V181.

Approximately 200 subjects will be randomized into the trial to receive a subcutaneous injection of either active vaccine or placebo administered at Day 1 and Month 6. As shown in Table 1, two formulations of V181 will be used in this trial:

- TV003 [rDENVlΔ30 (a dengue 1 vaccine); rDENV2/4Δ30(ME) (a dengue 2 vaccine); rDENV3Δ30/31 (a dengue 3 vaccine); and rDENV4Δ30 (a dengue 4 vaccine); similar concentrations for each component]
- TV005 [rDENVlΔ30 (a dengue 1 vaccine); rDENV2/4Δ30(ME) (a dengue 2 vaccine); rDENV3Δ30/31 (a dengue 3 vaccine); and rDENV4Δ30 (a dengue 4 vaccine); similar concentrations for all components except rDENV2/4Δ30(ME) which has 10-fold higher concentration]

Leibovitz L-15 medium will be utilized for the placebo and as diluent for field mix formulation. An unblinded pharmacist or qualified trial site personnel will be required at each site to field mix the study vaccine/placebo in order to maintain the blinding of the study.

Prior to receiving any study-related injections, subjects will be screened to document general good health. All vaccinated subjects will be followed for all adverse events for 28 days following each injection, beginning with the day of vaccination. Adverse events will be recorded using a standardized electronic Vaccination Report Card (eVRC) for the 28-day postvaccination safety follow-up period. In addition, all subjects will be followed for dengue-related adverse events (regardless of seriousness) including laboratory-confirmed dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS), all serious adverse events, and deaths due to any cause, for the time period beginning at informed consent through 1 year following the last vaccination.

For subjects who report fever of ≥38.0°C (≥100.4°F) for at least 2 consecutive days, investigators will conduct a clinical evaluation using 2009 World Health Organization (WHO) guidelines (Section 12.4) [1] to assess for dengue and clinical severity, and collect a serum specimen to test for dengue. For the V181 program, a case of virologically-confirmed dengue (VCD) is defined as fever of ≥38.0°C (≥100.4°F) for at least 2 consecutive days with a positive qualitative and/or quantitative serotype-specific reverse transcription polymerase chain reaction (RT-PCR) assay (Section 7.1.3.3).

An interim analysis (IA) is planned to evaluate immunogenicity and safety data at 28 days Postdose (PD) 1 to support internal decision making. Information regarding the IA and trial blinding can be found in Sections 5.2.3, 8.2, and 8.7.

This study will utilize an unblinded Standing Internal Data Monitoring Committee (siDMC) to facilitate early detection of any safety signals from initiation of the study to release of the IA to the Merck headquarter personnel. Additional information regarding the role of the siDMC is in Section 7.3.1.

Blood samples for the assessment of immune responses will be collected from all subjects at 7 time points, as outlined in Section 4.2.3.2. Details for all laboratory testing for this trial, including immunogenicity, viremia, acute illness testing, and safety testing are found in Section 7.1.3.

Specific procedures to be performed during the trial, as well as their prescribed times and associated visit windows, are outlined in the Trial Flow Chart - Section 6.0. Details of each procedure are provided in Section 7.0 – Trial Procedures.

2.2 Trial Diagram

The study trial design is depicted in Figure 1 and Figure 2.

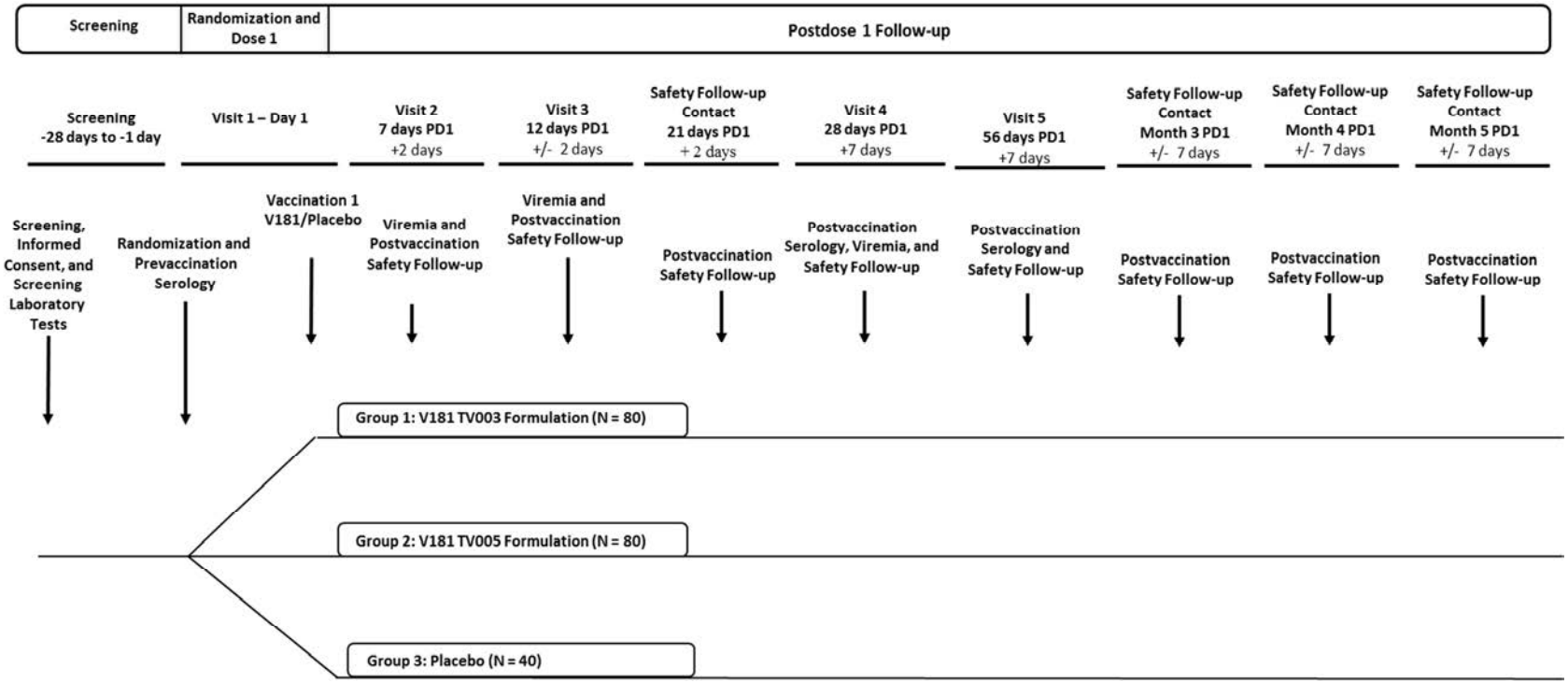
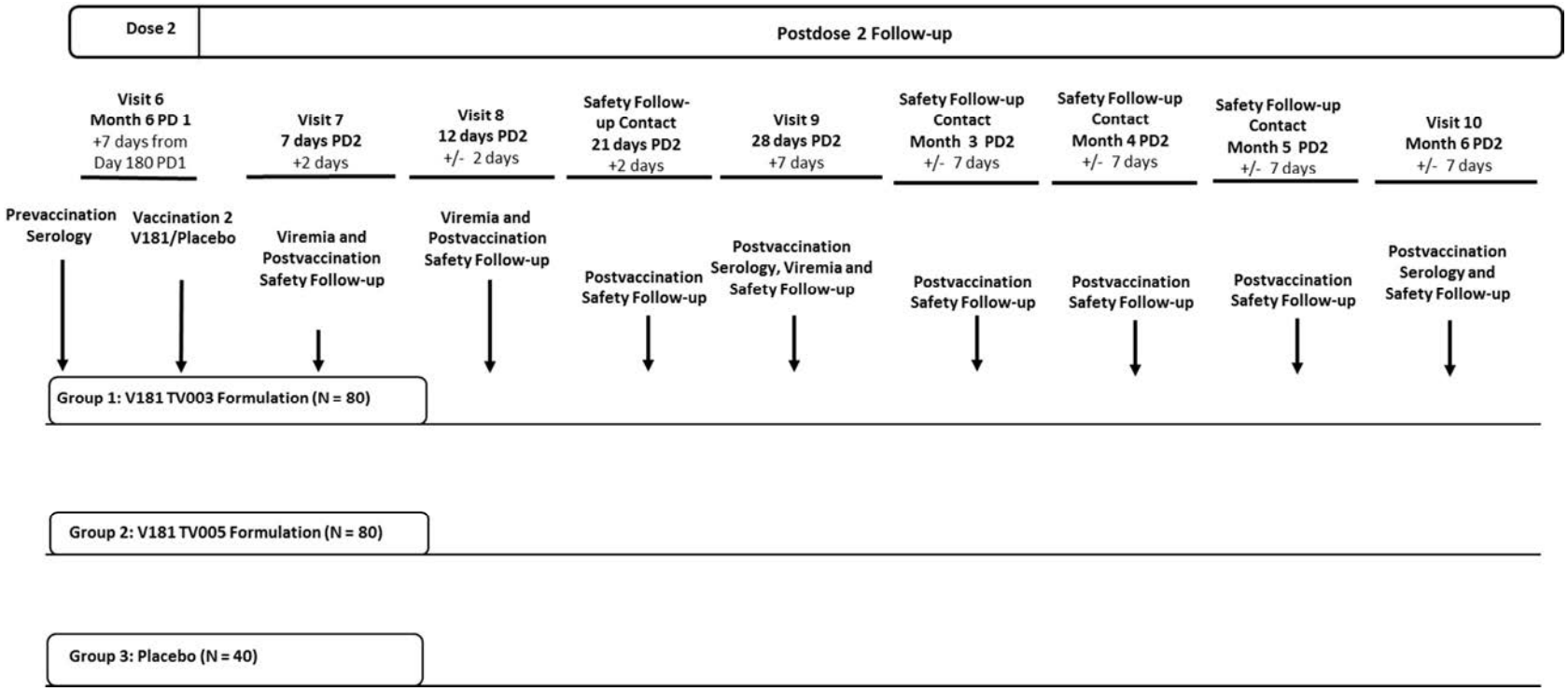


Figure 1 Trial Design for Screening Through Postdose 1 Follow-up


Dose 2
Postdose 2 Follow-up
Visit 6
Month 6 PD 1
+7 days from
Day 180 PD1
Visit 7
7 days PD2
+2 days
Visit 8
12 days PD2
+/- 2 days
Safety Follow-
up Contact
21 days PD2
+2 days
Visit 9
28 days PD2
+7 days
Safety Follow-up
Contact
Month 3 PD2
+/- 7 days
Safety Follow-up
Contact
Month 4 PD2
+/- 7 days
Safety Follow-up
Contact
Month 5 PD2
+/- 7 days
Visit 10
Month 6 PD2
+/- 7 days
Prevaccination
Serology
Vaccination 2
V181/Placebo
Viremia and
Postvaccination
Safety Follow-up
Viremia and
Postvaccination
Safety Follow-up
Postvaccination
Safety Follow-up
Postvaccination
Serology, Viremia and
Safety Follow-up
Postvaccination
Safety Follow-up
Postvaccination
Safety Follow-up
Postvaccination
Safety Follow-up
Postvaccination
Serology and
Safety Follow-up
Group 1: V181 TV003 Formulation (N = 80)
Group 2: V181 TV005 Formulation (N = 80)
Group 3: Placebo (N = 40)

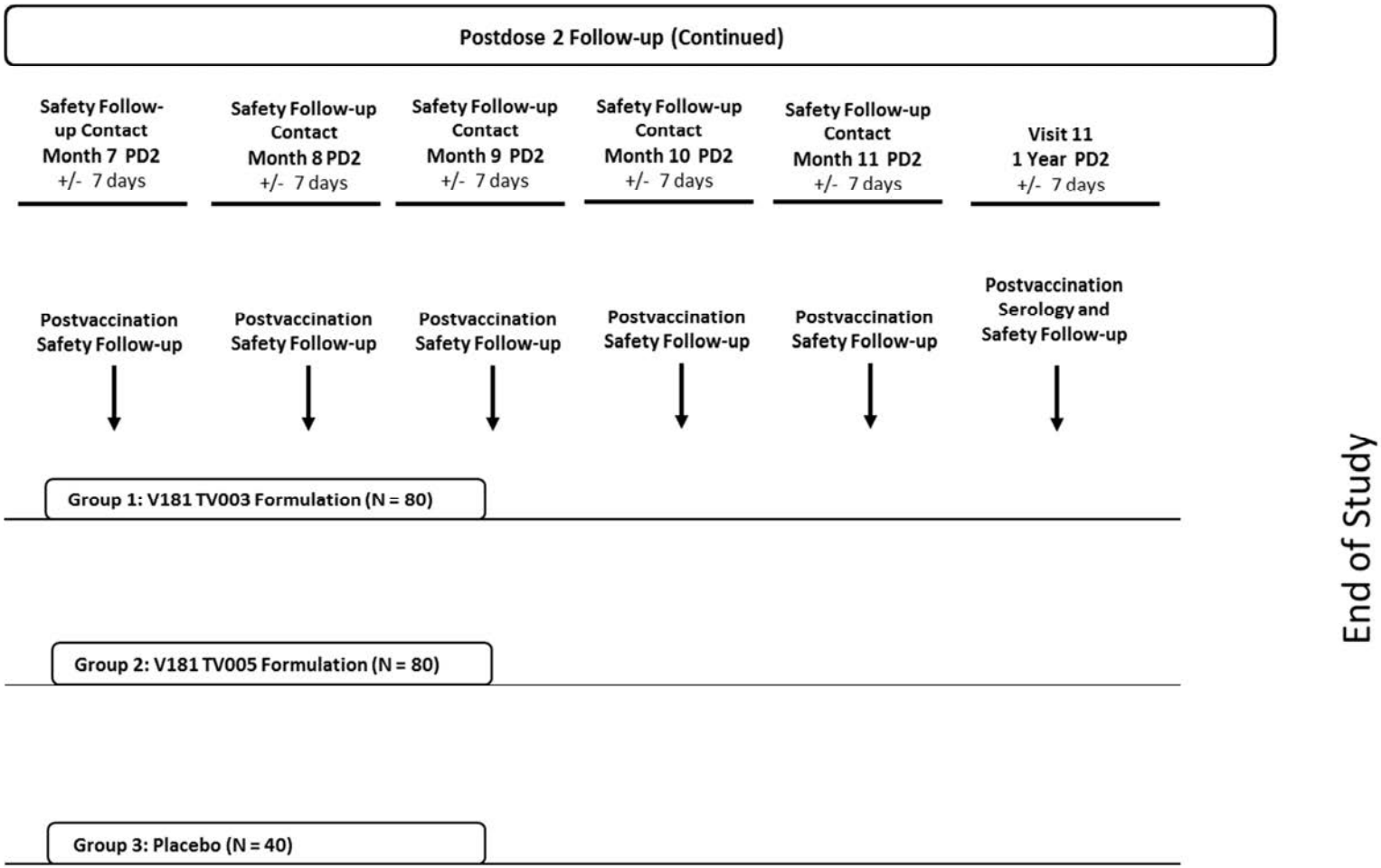


Figure 2 Trial Design for Dose 2 Through End of Study

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

In healthy male and female subjects 18 to 50 years of age:

3.1 Primary Objective(s) & Hypothesis(es)

Objective: To evaluate the overall safety and tolerability of the tested V181 formulations. No formal hypothesis will be tested.

3.2 Secondary Objective(s) & Hypothesis(es)

Objective: To evaluate the percentage of individuals who are seropositive by Virus Reduction Neutralization Test (VRNT) at 28 days PD1, separately for each serotype. Seropositivity is defined as having VRNT above the lower limit of detection of the assay [titers ≥10]. No formal hypothesis will be tested.

3.3 Exploratory Objectives

1) **Objective:** To evaluate the geometric mean titer (GMT) of virus-neutralizing antibodies measured by VRNT titer separately for each serotype at each immunogenicity assessment time point (Section 4.2.3.2).
2) **Objective:** To evaluate the geometric mean fold ratios (GMFR) of virus-neutralizing antibodies measured by VRNT titer separately for each serotype at 28 days post each vaccination compared to prevaccination (Study Day 1 or Study Month 6 for Dose 1 and Dose 2, respectively).
3) **Objective:** To evaluate the percentage of individuals who are seropositive by VRNT titers ≥10, separately for each serotype, at each immunogenicity assessment time point other than 28 days PD1.
4) **Objective:** To evaluate the percentage of individuals with detectable viremia (measured by polymerase chain reaction [PCR]) for any serotype at 7, 12, and 28 days following each vaccination.

4.0 BACKGROUND & RATIONALE

4.1 Background

Refer to the Investigator's Brochure (IB) for detailed background information on V181.

4.1.1 Pharmaceutical and Therapeutic Background

4.1.1.1 Clinical Findings of Dengue Infection

There are 4 serotypes of dengue viruses: DENV1, DENV2, DENV3, and DENV4. Infection with dengue viruses leads to a diverse clinical picture ranging from an in apparent or mild febrile illness, to classic DF characterized by high fever, headache, joint and muscle pain, rash, lymphadenopathy, and leukopenia, to life-threatening DHF and DSS. The clinical picture of DHF and DSS is characterized by hemorrhagic manifestations ranging from the presence of petechiae and ecchymosis to spontaneous severe hemorrhage and profound shock which may, if untreated, result in death. These more severe forms of the disease occur most

often after secondary dengue infection, when infection with 1 serotype of dengue virus is followed some time later by a second infection with another serotype. The severity of secondary dengue infections has been observed to increase with a longer interval between the first and second infection [2].

Although DF is less severe than DHF/DSS, a substantial burden of illness is associated with DF. The burden of illness associated with non-hospitalized dengue compared with severe hospitalized cases has been studied in Thailand, and it was found that between 44% and 73% of the disability-adjusted life-years (DALYs) are attributable to non-hospitalized cases [3]. The impact of the overall burden of clinical dengue disease, as opposed to DHF/DSS alone, is probably even more important in Latin America [4], where there are very large numbers of DF cases and a large economic impact. These data suggest that prevention of any dengue disease (DF or DHF/DSS) will be critical from a health economics perspective.

4.1.1.2 Unmet Medical Need and Epidemiology

There is a large unmet medical need for dengue vaccines. Dengue is among the most important arthropod-borne viral diseases in terms of human morbidity and mortality [5] [6] [7] [8] [3] [9] [10] [11] [12]. Dengue is endemic in more than 140 countries in Africa, the Americas, the Eastern Mediterranean, and Asia, and strains of each dengue serotype are found circulating throughout these regions. It is currently estimated that there are up to 4 billion people who live in tropical and subtropical countries where dengue transmission occurs, and who therefore are at risk for infection[13] [14]. Each year, dengue viruses are estimated to infect approximately 390 million people throughout the tropics and subtropics, causing an estimated 96 million symptomatic cases with 2.1 million cases of severe disease, and approximately 50,000 deaths [14] [15] [16].

The epidemiology of dengue infection is complex since the virus affects all age groups and the burden of disease by age group varies by geography, immunological status of the host, and circulating serotypes. For example, in the Americas, the highest incidence of DF and DHF is generally among adolescents and adults, with a recent shift in the last few years to younger age groups in select countries [17]. In contrast, in Asia, DHF primarily affects younger children [3] [9] [10]. Currently, no antiviral treatments exist and future hopes for control rely on the development of improved vector control and an effective vaccine.

4.1.1.3 Immunologic Response to Natural Dengue Infection

Primary dengue virus infection results in the induction of virus-neutralizing antibodies that are broadly cross-reactive early after infection, and become more type-specific over time. Primary dengue infection confers long-lasting immunity to the infecting serotype, but only short-term protection against the other dengue serotypes.

In contrast, infection with a second dengue virus type typically results in very robust and broadly cross-reactive immune responses, including high-titer virus-neutralizing antibodies reactive against all 4 virus types [18] [19]. Disease associated with third or fourth dengue virus infection is only rarely reported, suggesting that most individuals are protected against all virus serotypes following secondary infection [20].

4.1.1.4 Hypothesized Immune Mechanisms Causing DHF and DSS

DHF and DSS occur most often after secondary dengue infections, when infection with 1 dengue serotype is followed some time later by a second infection with another serotype. The more frequent association of DHF and DSS with secondary dengue infection is hypothesized to be mediated by immune mechanisms [21]. This is supported by the fact that DHF occurs relatively late in infection (at the time of defervescence), when the virus has disappeared from the blood. Studies have suggested that the plasma leakage syndrome associated with DHF is linked to the release of cytokines [21].

4.1.1.5 Dengue Vaccine Development

As stated previously, given the possible role of immune responses in more severe disease, vaccine approaches must be carefully considered. The major approach for mitigating the risk of vaccine-induced sensitization for DHF is development of a tetravalent vaccine that will simultaneously and durably protect against disease caused by all of the 4 dengue serotypes [22]. Due to the theoretical risk for enhancement of subsequent dengue disease following vaccination, long-term monitoring for dengue disease is required for vaccine studies conducted in dengue-endemic areas.

Currently, Sanofi Pasteur is the only vaccine manufacturer that has received approval for a dengue vaccine. As of December 2016, Sanofi's dengue vaccine, Dengvaxia™, has been approved in 11 countries (Mexico, the Philippines, Brazil, El Salvador, Costa Rica, Paraguay, Guatemala, Peru, Indonesia, Thailand, and Singapore). The indication is for persons 9 to 45 years of age living in endemic areas. [23]

There are 2 reasons to believe that V181 LATV can be successful:

- In Phase 1 clinical trials, the National Institutes of Health (NIH) LATV formulation generated a balanced neutralizing-antibody response, which supports a reasonable probability of success for achieving tetravalent efficacy.
- Live-attenuated and inactivated vaccines for other flaviviruses (yellow fever, Japanese encephalitis, and tick-borne encephalitis) are licensed and highly efficacious.

4.1.1.5.1 Merck Live-Attenuated Tetravalent Vaccine (V181)

Live-attenuated tetravalent vaccine has been developed in the Laboratories of Infectious Disease (LID) of the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH. The NIH spent more than 10 years designing the individual vaccine components and demonstrating safety and immunogenicity of the monovalent vaccine candidates in completed nonclinical and Phase 1 clinical trials. After defining the individual components they launched clinical testing of tetravalent formulations in flavivirus-naïve populations, including studies that showed the tetravalent vaccine protected against subsequent challenge with dengue. Live-attenuated tetravalent vaccine is being studied in flavivirus-experienced populations in Thailand and Bangladesh by the NIAID team and in Brazil by the Instituto Butantan. Instituto Butantan is currently conducting a Phase 3 clinical trial.

The final vaccine has evolved to a composition of 4 viral components representing each of the 4 dengue serotypes, DENV1, DENV2, DENV3 and DENV4. Attenuation of all 4 viral components in TV003 and TV005 was achieved by genetic modification, i.e., deletion of 30 nucleotides in the 3' non-coding region (Δ30) of the dengue genome. DENV2 is the only serotype that is not a full-length homotypic genome but is instead a chimeric virus with the Pre-M and E protein from DENV2 inserted into an attenuated DENV4 backbone. In addition, DENV3 also has an additional 31-nucleotide deletion in the 3 non-coding region (Δ30/31). The vaccine viral strains are referred to as rDENV1Δ30, rDENV2/4Δ30(ME), rDENV3Δ30/31, and rDENV4Δ30 for DENV 1, 2, 3, and 4 respectively. All of the final vaccine strains have been fully characterized and their attenuation confirmed through in vitro and in vivo testing.

The monovalent and tetravalent vaccines were generally well tolerated in studies conducted by the NIH [24] [25] [26] [27]. Briefly, no subjects met the protocol-specified definition for dengue-like syndrome in the NIH sponsored trials and injection-site adverse events were generally mild and self-limited. An asymptomatic rash occurs at higher rates among vaccinees than placebo recipients after the first dose, and was generally not observed after the second dose. This dengue vaccine-associated rash included scattered macular/maculo-papular lesions. It was typically found on the trunk and proximal extremities and was most frequently seen 10 to 16 days after vaccination. In clinical trials, viremia was monitored and the peak level of viremia remained low. Transient asymptomatic neutropenia has been observed in vaccinees in some studies and was typically associated with a baseline neutrophil count $\leq 3000/mm^3$ in subjects of African descent. Generally no other AEs reached statistical significance in vaccinees compared to placebo in the NIH-sponsored studies.

The key immunogenicity findings from the NIH-sponsored trials include that following a single dose of LATV administered to flavivirus-naïve subjects, seroconversion to 3 or 4 dengue types is observed in ≥90% of subjects. The proportion who seroconverted to all 4 types ranged from approximately 45% to 90%, depending on the formulation and time frame considered. Seroconversion rates increased following a second tetravalent vaccine dose given 6 months after the first dose. However, Postdose 2- (PD2) fold rises in mean peak titers for all DENV types were ≤2.1-fold, and the mean peak titers were lower PD2 than PD1. The NIH attributed this observation to inhibition of viral replication due to sterilizing immunity following the first vaccine dose, to reinfection with homologous vaccine virus.

For this Phase 1 study, the LATV dengue vaccine candidates TV003 and TV005 will be studied. TV003 and TV005 are admixtures of the same 4 recombinant monovalent DENV vaccines. Each component of the vaccine is administered at a dose of 1,000 plaque-forming units (PFUs) with the exception of the rDENV2/4Δ30(ME) component in TV005, which is administered at a dose of 10,000 PFU. In NIH-sponsored clinical trials, subjects received 2 doses of the vaccine (or placebo) 6 months apart to both evaluate the safety of the vaccine and the ability of a second dose of vaccine given 6 months later to boost the antibody response. The admixtures were evaluated in flavivirus-naïve healthy adult subjects.

The NIH-sponsored nonclinical and clinical trials have shown that the tetravalent vaccine formulations are generally well tolerated and that the TV003 and TV005 formulations show robust and well balanced immunogenicity in flavivirus-naïve populations with a majority of

subjects developing a tetravalent or trivalent response after a single dose. In two Phase 1 clinical trials, a single dose of TV005 elicited a tetravalent response in 90% of vaccine recipients 3 months after vaccination and a trivalent response in 98% of vaccine recipients. In these early studies, TV005 elicited improved seroconversion frequencies and overall antibody titers to DENV2 compared to TV003. In addition, the studies have shown that administration of a second dose is also well tolerated with a modest impact on immunogenicity, primarily acting as a catch up for those subjects that did not develop trivalent or tetravalent responses to the first dose. Currently TV003 and TV005 are being studied in both flavivirus-naïve populations in the United States and flavivirus-experienced populations in the United States, Thailand, and Bangladesh (NIH-sponsored clinical trials). TV003 is also being studied in Brazil by the Instituto Butantan in a large Phase 3 clinical trial (N = ~17,000 subjects). Overall, more than 500 subjects have received vaccination with a monovalent component of the tetravalent vaccine and over 500 subjects have received a tetravalent vaccine formulation in the NIH studies.

4.1.2 Pre-clinical and Clinical Trials

4.1.2.1 Pre-clinical Trials

Preclinical safety studies of LATV have been conducted by the NIH. Given that the NIH LATV and the Merck LATV are similar by design, the existing preclinical trial data with the NIH LATV will support this Phase 1 clinical trial. Details of the pre-clinical trials of the NIH LATV are outlined in the Investigator's Brochure (IB).

4.1.2.2 Ongoing Clinical Trials

There have been no clinical trials for V181 conducted by Merck as the Sponsor to date. Given that the NIH LATV and the Merck LATV are similar by design, the existing completed clinical trials with NIH LATV will support this Phase 1 clinical trial. A summary of ongoing and/or completed clinical trials of the NIH LATV are provided in the IB.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

The study will provide initial information on the safety, tolerability, and immunogenicity of LATV manufactured by Merck, from Merck-generated master virus seeds. There are no clinically significant differences expected between the Merck and NIH formulations of LATV based on sequencing information and similarity of manufacturing. Therefore, the purpose of this Phase 1 study is to assess the safety and tolerability of the V181 formulations (TV003 and TV005) and determine if the formulations can produce neutralizing-antibody responses to DENV1, DENV2, DENV3, and DENV4 at 28 days PD1 in both flavivirus-naïve and flavivirus-experienced subjects.

The sample size of 200 subjects was chosen based on the estimated confidence interval widths as outlined in Section 8.9. This sample size is considered meaningful from a safety perspective, which is the primary objective of this study.

Clinical trial sites in the continental United States and Puerto Rico were selected based on the dengue seroprevalence rate in order to enroll both seronegative and seropositive subjects. Based on epidemiology data, Puerto Rico sites will enroll the majority of the seropositive subjects, because the seroprevalence rate is approximately 85% [28].

4.2.2 Rationale for Dose Selection/Regimen

A single dose of V181 or placebo will be administered subcutaneously at Day 1 and a second dose of V181 or placebo will be administered at Month 6. The 2-dose regimen and 6-month interval between doses were selected based on the NIH Phase I clinical trial data. The NIH Phase 1 clinical trial data demonstrated administration of a second dose of V181 is well tolerated with a modest impact on immunogenicity. The second dose is thought to act as a catch up for those subjects that did not develop trivalent or tetravalent responses to the first dose [25] [26]. As previously discussed, it is important to develop a vaccine that has a well-balanced, durable tetravalent response to all 4 dengue serotypes in order to mitigate the risk of vaccine-induced sensitization for DHF [29].

TV003 and TV005 will be used in this study in order to obtain additional information regarding the differences in immune responses between these 2 formulations. As mentioned in Section 4.1.1.5.1, NIH has conducted several studies using each of these formulations. In two Phase 1 clinical trials, a single dose of TV005 elicited a tetravalent response in 90% of vaccine recipients 3 months after vaccination and a trivalent response in 98% of vaccine recipients. In these early studies, TV005 elicited improved seroconversion frequencies and overall antibody titers to DENV2 compared to TV003. This Phase 1 study (V181-001) will provide the opportunity to see if similar results are seen using the LATV manufactured from the Merck-generated master virus seeds.

In addition, the dose level was also chosen based upon previous clinical trials conducted by the NIH. There is no dose modification in this study.

4.2.2.1 Rationale for the Use of Placebo

Placebo (Leibovitz L-15 medium) will be administered as 1 arm of this trial (N=40) in order to preserve the blinding and provide a control for the active study vaccine for safety assessments. The Leibovitz L-15 medium was chosen as the placebo in order to be consistent with the placebo used in the NIH-sponsored clinical trials. This medium was also selected because it can be used as both the placebo and the diluent for the field mix of the active formulations (TV003 and TV005).

4.2.3 Rationale for Endpoints

4.2.3.1 Safety Endpoints

The safety observation period planned is 28 days after each dose using an eVRC for solicited/unsolicited systemic and local reactions.

Specific injection-site adverse events (injection-site pain, injection-site erythema, and injection-site swelling) will be solicited 1 to 5 days following each vaccination. Systemic

adverse events, unsolicited injection-site adverse events, and daily measurement of oral temperature, will be reported from 1 to 28 days following each vaccination. For this study, temperatures ≥38.0°C (≥100.4°F) oral or equivalent will be considered a fever. Prespecified solicited systemic adverse events, which include rash, fatigue (tiredness), malaise (feeling sick), headache, and myalgia (muscle ache) will also be collected for 28 days postvaccination. Rash reported from 1 to 28 days following each vaccination will be specifically assessed to determine if the rash is either a dengue-like rash or a dengue vaccine-associated rash (see Section 7.1.2.5 for more details).

The 28-day safety period was chosen based on the safety profile that was observed in NIH clinical trials of LATV to date. During this period, subject visits will occur on 7, 12, and 28 days following each vaccination for safety follow-up, including safety laboratory measurements and qualitative and/or quantitative RT-PCR for the detection of vaccine virus viremia. These time points were chosen for consistency with the observations in the previous studies (i.e., asymptomatic rash, viremia, and other significant safety findings). All dengue-related adverse events (defined as laboratory-confirmed DF, DHF, and/or DSS regardless of seriousness), serious adverse events, and deaths that occur for the time period beginning at informed consent through 1 year following the last vaccination are to be reported.

Subjects who report fever of ≥38.0°C (≥100.4°F) for at least 2 consecutive days will be instructed to call the study site as soon as possible to schedule an urgent acute office visit for clinical assessment and serum collection. This acute initial evaluation visit should occur as close as possible to the second day of fever, and no later than 3 days after the second day of protocol-specified fever. Investigators will conduct a clinical evaluation using 2009 WHO guidelines (Section 12.4) [1] to assess for dengue and clinical severity, and collect a serum specimen to test for dengue.

Virologically-confirmed dengue is defined as 2 or more consecutive days of fever ≥38.0°C (≥100.4°F) with a positive qualitative and/or quantitative serotype-specific RT-PCR assay result.

4.2.3.2 Rationale for Immunogenicity Measurements and Assay Methods

The ability of the V181 formulations (TV003 / TV005) to induce neutralizing antibodies to the 4 vaccine serotypes 28 days PD1 will be assessed in this study using the VRNT assay. Antibody responses will be measured in the entire study population (N=200) at the following time points as shown in the Trial Flow Chart (Section 6.0).

1. Visit 1 (baseline), Day 1, prior to vaccination with Dose 1
2. Visit 4, 28 days PD1
3. Visit 5, 56 days PD1
4. Visit 6, Month 6 PD1, prior to vaccination with Dose 2
5. Visit 9, 28 days PD2
6. Visit 10, Month 6 PD2
7. Visit 11, Year 1 PD2

The time point of 28 days was chosen based on the immunogenicity results seen in the NIH clinical trials to date. Additional immunogenicity time points will be assessed (see Study Flow Chart in Section 6.0 for list of all time points that samples for immunogenicity are collected). Seropositivity is defined as having VRNT above the lower limit of detection of the assay [titers ≥10]. A threshold of ≥10 was defined based upon the technical performance of the assay. While no correlate of protection has been established for dengue, the measurement of virus-neutralizing antibody responses provides a mechanism to assess the immunogenicity of the vaccine using a functional assay that may predict the potential for protection.

4.2.3.3 Future Biomedical Research

The Sponsor will conduct Future Biomedical Research on specimens consented for future biomedical research during this clinical trial. This research may include genetic analyses (DNA), gene expression profiling (RNA), proteomics, metabolomics (serum, plasma) and/or the measurement of other analytes, depending on which specimens are consented for future biomedical research.

Such research is for biomarker testing to address emergent questions not described elsewhere in the protocol (as part of the main trial) and will only be conducted on specimens from appropriately consented subjects. The objective of collecting/retaining specimens for Future Biomedical Research is to explore and identify biomarkers that inform the scientific understanding of diseases and/or their therapeutic treatments. The overarching goal is to use such information to develop safer, more effective drugs/vaccines, and/or to ensure that subjects receive the correct dose of the correct drug/vaccine at the correct time. The details of this Future Biomedical Research sub-trial are presented in Section 12.2 – Collection and Management of Specimens for Future Biomedical Research.

4.3 Benefit/Risk

Subjects in clinical trials generally cannot expect to receive direct benefit from treatment/vaccination during participation, as clinical trials are designed to provide information about the safety and effectiveness of an investigational medicine.

Additional details regarding specific benefits and risks for subjects participating in this clinical trial may be found in the accompanying IB and Informed Consent documents.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Healthy male and female subjects between 18 and 50 years of age (inclusive) will be enrolled in this trial.

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be in good health, based on medical history and physical examination.
2. Be 18 to 50 years of age upon receipt of the first study vaccination.
3. Understand the study procedures, alternative treatments available (if applicable), and risks involved with the study, and voluntarily agree to participate by giving written informed consent. The subject may also provide consent/assent for Future Biomedical Research; however, the subject may participate in the main trial without participating in Future Biomedical Research.
4. Be able to read, understand, and complete study questionnaires (i.e., the Vaccination Report Card).
5. Be able to complete all scheduled visits including non-office visit contacts (e.g., telephone) and to comply with the study procedures.
6. Agree to avoid unusual, unaccustomed strenuous, vigorous physical exercise/activity (e.g., beginning new weight-lifting, running, or bicycling regimens) from 72 hours before a given dose of study vaccine/placebo through 28 days following that dose.
7. Be highly unlikely to conceive at any time from signing the informed consent through 4 weeks after receiving the last dose of the study vaccine/placebo, as indicated by at least one "yes" answer to the following questions:

   a) The subject is a male who is not of reproductive potential, defined as a male who has azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

   b) The subject is a female who is not of reproductive potential, defined as a female who either: (1) is postmenopausal (defined as at least 12 months with no menses in women ≥45 years of age); (2) has had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy, or bilateral tubal ligation/occlusion at least 6 weeks prior to screening; OR (3) has a congenital or acquired condition that prevents childbearing.

   c) The subject is a female or a male who is of reproductive potential and agrees to avoid becoming pregnant or impregnating a partner for 4 weeks following the last study vaccination by complying with one of the following: (1) practice abstinence† from heterosexual activity OR (2) use (or have their partner use) acceptable contraception during heterosexual activity. Acceptable methods of contraception are‡:

      Single method (one of the following is acceptable):

      - Non-hormonal intrauterine device (IUD)
      - Vasectomy of a female subject's male partner
      - Contraceptive rod implanted under the skin

Combination method (requires use of two of the following):

- Diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- Cervical cap with spermicide (nulliparous women only)
- Contraceptive sponge (nulliparous women only)
- Male condom or female condom (cannot be used together)
- Hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection.

[†]Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and Ethics Review Committees (ERCs)/Institutional Review Boards (IRBs). Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

[‡]If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

For items with an asterisk (*), if the subject meets these exclusion criteria, the Day 1 Visit may be rescheduled for a time when these criteria are not met.

1. Has a history of receiving any investigational flavivirus vaccine.
2. Has a known hypersensitivity to any component of the dengue vaccine, or history of severe allergic reaction (e.g., swelling of the mouth and throat, difficulty breathing, hypotension or shock) that required medical intervention.
3. Abused drugs or alcohol within 12 months prior to screening which, in the opinion of the investigator, may interfere with the evaluations required by the study or with completion of the study.
4. Is pregnant or breastfeeding, or expecting to conceive at any time from signing the informed consent through 4 weeks after receiving the last dose of the study vaccine/placebo.
5. Has 1 or more of the following screening laboratory values **and** is considered clinically significant by the investigator:
    a. Alanine aminotransferase (ALT) ≥1.25 times the upper limit of normal (ULN[†]).
    b. Positive urine glucose or urine protein >1+ by dipstick or urinalysis.

c. Estimated glomerular filtration rate (eGFR) < lower limit of normal (LLN†) by gender.

d. Hematology results as follows:

- Hemoglobin < the lower limit of normal (LLN†)
- White blood cells (WBC) < LLN* or > ULN†
- Platelets < LLN†

†See Central Laboratory's Lab Manual for specific LLN and ULN for individual tests.

6. Has a positive serum test for human immunodeficiency virus (HIV) antibody, hepatitis B surface antigen (HBsAg), and/or hepatitis C antibody.
7. Has known, suspected, or a history of immunocompromise, including congenital immunodeficiency, HIV infection, leukemia, lymphoma, Hodgkin's disease, multiple myeloma, generalized malignancy, chronic renal failure (most recent serum creatinine values in medical record ≥3 mg/dL), nephrotic syndrome, or other conditions associated with immunosuppression, including ever receiving an organ or bone marrow transplant.
8. Has a history of malignancy ≤5 years prior to signing informed consent (other than adequately treated non-melanoma skin cancer).
9. Has poorly controlled diabetes mellitus, is receiving insulin or an oral antidiabetic agent, and has a glycosylated hemoglobin (HbA1c) level ≥9% (≥3.5% ULN) tested within 4 months prior to screening.

   Note: For those subjects who report a medical history of diabetes mellitus during screening, and cannot provide an HbA1c level within 4 months prior to screening, perform HbA1c level to assess whether subject is excluded from the study based upon this criterion.
10. Uses any immunosuppressive therapy (Note: topical and inhaled/nebulized steroids are permitted). Subjects on corticosteroids or who may receive corticosteroids (e.g., asthmatics) should be excluded if they are receiving or are expected to receive, at any time from signing the informed consent through 1 year after receiving the last dose of the study vaccine/placebo, systemic doses greater than required for physiological replacement, i.e., >5 mg of prednisone (or equivalent) daily and for >2 weeks. Immunosuppressive therapies which also meet the exclusion criteria include chemotherapeutic agents used to treat cancer or other conditions, and treatments associated with organ or bone marrow transplantation, or autoimmune disease.
11. * Subject has received a licensed non-live vaccine within 14 days prior to receipt of the first dose of study vaccine/placebo. (Exception: Inactivated influenza vaccine may be given at least 7 days prior to receipt of the study vaccine/placebo.)
12. * Has received a licensed live vaccine within 28 days prior to receipt of the first dose of study vaccine/placebo.

13. * Has received investigational drugs or vaccines within 2 months prior to receipt of first dose of study vaccine/placebo.
14. Has a history of ever receiving 1 or more doses of an investigational or licensed dengue vaccine.
15. Has participated in another interventional clinical study within 2 months prior to signing the informed consent, or planned enrollment in another interventional clinical study at any time from signing the informed consent through 1 year after receiving the last dose of the study vaccine/placebo.
16. Received a blood transfusion or blood products (including immune globulin) within 6 months prior to receipt of first dose of study vaccine/placebo.
17. Has made donation or phlebotomy of >300 mL of blood products within 8 weeks prior to signing the informed consent, or planned donation or phlebotomy of >300 mL of blood products outside of the study at any time from signing the informed consent through 28 days after receiving the last dose of the study vaccine/placebo.
18. Has planned donation of eggs or sperm at any time from signing the informed consent through 28 days after receiving the last dose of the study vaccine/placebo.
19. *Had recent hospitalization for acute illness within the 3 months prior to receipt of the first dose of study vaccine/placebo.
20. * Has a history of febrile illness (≥38.0°C [≥100.4°F] oral or equivalent) occurring within 72 hours prior to receipt of the first dose of study vaccine/placebo.
21. Has a condition in which repeated venipuncture or injections pose more than minimal risk for the subject, such as hemophilia, thrombocytopenia, other severe coagulation disorders, or significantly impaired venous access.
22. Has major psychiatric illness including: any history of schizophrenia or severe psychosis, bipolar disorder requiring therapy, or any subject with suicidal ideation within 3 years prior to receipt of the first dose of study vaccine/placebo.
23. Has previously been enrolled into this study and subsequently withdrawn.
24. Is unlikely to adhere to study procedures, keep appointments, or is planning to relocate during the study.
25. Has any other underlying medical condition or reason that, in the opinion of the investigator, may interfere with the evaluations required by the study or with completion of this study.
26. Is or has an immediate family member (e.g., spouse, parent/legal guardian, sibling or child) who is investigational site or Sponsor staff directly involved with this trial.

5.1.3.1 Subject Deferment Criteria (Prior to Vaccination 2)

These subject deferment criteria must be reviewed for each subject prior to vaccination 2 to ensure that none of the criteria apply to the subject, and/or that vaccination 2 is deferred for the subject for an appropriate period of time.

The subject should be deferred from continuing in the trial if the subject:

1. Has had recent (within 72 hours) history of febrile illness (≥38.0°C [≥100.4°F] oral equivalent) (defer second dose until >72 hours after resolution of febrile illness).
2. Received inactivated influenza vaccine within 7 days prior to scheduled receipt of study vaccine /placebo. Defer second dose until a minimum of 8 days after receipt of inactivated influenza vaccine.
3. Has had any medical condition that, in the opinion of the investigator, may interfere with the evaluation of the study objectives. (The amount of time the second dose is deferred will be determined by the investigator.)

5.1.3.2 Criteria for Excluding Subjects from Receiving Vaccination 2

These subject criteria must be reviewed for each subject prior to vaccination 2 to ensure that none of the criteria apply to the subject.

The subject must be excluded from receiving vaccination 2 if the subject:

1. Is pregnant; has had a positive pregnancy test without resolution of the pregnancy prior to vaccination 2 visit; has not been compliant with contraception methods outlined in inclusion criterion 7; or plans to become pregnant within 4 weeks following vaccination 2.

2. Has received one or more of the concomitant medications or vaccinations outlined in Section 5.5 (Concomitant Medications/Vaccinations [Allowed & Prohibited]).

3. Has enrolled in another interventional clinical study or received another flavivirus or dengue vaccine since signing the informed consent.

4. Has had a vaccine-related allergic or anaphylactoid reaction reported following vaccination 1.

5. Has had, since vaccination 1, one or more of the following screening laboratory values **and** it is considered clinically significant by the investigator:

    a. ALT ≥1.25 times the upper limit of normal (ULN†).
    b. Positive urine glucose or urine protein >1+ by dipstick or urinalysis.
    c. Estimated glomerular filtration rate (eGFR) < lower limit of normal (LLN†) by gender.
    d. Hematology results as follows:
        - Hemoglobin < the lower limit of normal (LLN†)
        - White blood cells (WBC) < LLN† or > ULN†
        - Platelets < LLN†

    †See the Central Laboratory's Lab Manual for specific LLN and ULN for individual tests.

6. Had their treatment assignment unblinded by the investigator, Merck subsidiary, or through the emergency unblinding call center and they were randomized to receive placebo.
7. Has performed unusual, unaccustomed strenuous, vigorous physical exercise/activity (e.g., beginning new weight-lifting, running, or bicycling regimens) within 72 hours prior to vaccination 2, or does not agree to refrain from unusual, unaccustomed strenuous, vigorous physical exercise/activity through 28 days following vaccination 2.

5.2 Trial Vaccination(s)

The vaccine(s) to be used in this trial are outlined below in Table 1.

Table 1 Trial Vaccination

Vaccine	Dose/Potency	Dose Frequency	Route of Administration	Vaccination Regimen	Use
V181 TV003 Formulation	0.5 mL	2	subcutaneous	Visit 1 (Day 1) and Visit 6 (Month 6 PD1)	investigational vaccine
V181 TV005 Formulation	0.5 mL	2	subcutaneous	Visit 1 (Day 1) and Visit 6 (Month 6 PD1)	investigational vaccine
Placebo	0.5 mL	2	subcutaneous	Visit 1 (Day 1) and Visit 6 (Month 6 PD1)	placebo

Trial Vaccination (Dose 1) is given on the day of treatment allocation/randomization or as close as possible to the date on which the subject is allocated/assigned. Trial Vaccination (Dose 2) is given 6 months after Dose 1 was given.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of trial treatments in accordance with the protocol and any applicable laws and regulations.

5.2.1 Dose Selection

5.2.1.1 Dose Selection (Preparation)

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background & Rationale.

The final vaccine product, including placebo, will be a field mix prepared at the study site. An unblinded pharmacist or qualified trial site personnel will mix multiple study vaccine/placebo substances (i.e., each individual attenuated viral component and diluent) in order to produce the final study vaccine/placebo formulation that will be administered to the subject. The unblinded pharmacist and/or qualified trial site personnel will be provided with a Pharmacy Manual with the instructions for preparing the field-mixed formulations for all vaccination doses. Section 5.2.3 provides additional details around trial blinding.

5.2.2 Timing of Dose Administration

In this study, V181 or placebo will be administered as a 0.5-mL subcutaneous injection at Visit 1 (Day 1) and Visit 6 (Month 6 PD1). The day of the first study vaccination is considered Day 1 of the study. Study subjects will be observed by blinded study site personnel for 30 minutes following each vaccination for any immediate adverse events.

5.2.3 Trial Blinding

A triple-blind technique will be used to the IA release, and double-blind technique (site personnel and subjects remain blinded) to the end of the study. V181 and placebo will be prepared and/or dispensed in a blinded fashion by an unblinded pharmacist or qualified trial site personnel. The subject and the investigator who is involved in the vaccine administration or clinical evaluation of the subjects are unaware of the group assignments.

A triple-blind technique will be used to the IA release, and double-blind technique (site personnel and subjects remain blinded) to the end of the study. V181 and placebo will be prepared in an unblinded fashion by an unblinded pharmacist or qualified trial site personnel and administered in a blinded fashion by blinded site personnel. The subject and the investigator who is involved in the vaccine administration or clinical evaluation of the subjects will be blinded to the treatment group assignments.

An internal statistician and statistical programmer not directly involved in the conduct of the study will be unblinded from the initiation of the study to the release of the IA. After the IA is released, Merck Headquarters (HQ) personnel involved in the conduct of the trial will be unblinded. As such, the internal statistician and statistical programmer not directly involved with the conduct of the study will not be required after the IA.

The IA summary will display the aggregate immunogenicity and safety data for each vaccination group. All site personnel and participating subjects will remain blinded to the individual vaccination group assignments until the final study analysis is conducted at Year 1 PD2. Laboratory personnel will remain blinded to treatment group throughout the duration of the study.

See Section 7.1.4.2, Blinding/Unblinding, for a description of the method of unblinding a subject during the trial, should such action be warranted.

5.3 Randomization or Vaccine Allocation

Treatment allocation/randomization will occur centrally using an interactive response technology (IRT). There are 3 vaccination arms. Subjects will be assigned randomly in a 2:2:1 ratio to V181 TV003 Formulation, V181 TV005 Formulation, or placebo, respectively.

5.4 Stratification

Subjects will be stratified by geography (continental United States and Puerto Rico) to approximately balance the number of presumed flavivirus-seronegative and seropositive subjects who receive each formulation (TV003, TV005, and placebo) using an IRT. The randomization by vaccination group of 2:2:1 (TV003, TV005, and placebo) will remain the same within the continental United States and Puerto Rico.

Flavivirus serostatus is not a screening criteria; it will be used only to stratify the subjects for the subgroup analyses, as outlined in Section 8.10.

5.5 Concomitant Medications/Vaccinations (Allowed & Prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Sponsor Clinical Director. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the investigator, the Sponsor and the subject.

Listed below are specific restrictions for concomitant therapy or vaccination during the course of the trial:

1. Receipt of any investigational drugs or vaccines (other than provided study vaccines) within 2 months prior to receipt of first dose of study vaccine/placebo through 1 year PD2 (end of study) is prohibited.
2. Receipt of any licensed flavivirus vaccine, including dengue vaccine through 1 year PD2 (end of study) is prohibited.
3. Receipt of any immunosuppressive therapy (Note: topical and inhaled/nebulized steroids are permitted) from signing the informed consent through 1 year after receiving the last dose of the study vaccine/placebo (end of study) is prohibited.
4. Receipt of licensed non-live vaccine at any time from 14 days before receiving the first dose of study vaccine/placebo through 28 days after receiving the last dose of study vaccine/placebo is prohibited. (Exception: Inactivated influenza vaccine may be administered during the study, but must be given at least 7 days prior to receipt of the study vaccine/placebo or at least 15 days after receipt of the study vaccine/placebo.)
5. Receipt of licensed live vaccine at any time from 28 days before receiving the first dose of study vaccine/placebo through 28 days after receiving the last dose of the study vaccine/placebo is prohibited.
6. Receipt of a blood transfusion or blood products including immune globulin at any time from signing the informed consent through 28 days after receiving the last dose of the study vaccine/placebo is prohibited.
7. Planned enrollment in another interventional clinical study at any time from signing the informed consent through 1 year after receiving the last dose of the study vaccine/placebo (end of study) is prohibited.

5.6 Rescue Medications & Supportive Care

No rescue or supportive medications are specified to be used in this trial.

5.7 Diet/Activity/Other Considerations

Subjects should refrain from unusual, unaccustomed vigorous physical exercise/activity (e.g., beginning new weight-lifting, running, or bicycling regimen) for 72 hours prior and 28 days following each vaccination. Subjects should maintain a normal diet unless modifications are required to manage an adverse event such as diarrhea, nausea, or vomiting.

5.8 Subject Withdrawal/Discontinuation Criteria

5.8.1 Discontinuation of Vaccination

Discontinuation of vaccination does not represent withdrawal from the trial.

As certain data on clinical events beyond vaccination discontinuation may be important to the study, they must be collected through the subject's last scheduled follow-up, even if the subject has discontinued vaccination. Therefore, all subjects who discontinue trial vaccination prior to completion of the vaccination period will still continue to participate in the trial as specified in Section 6.0 and Section 7.1.5.3.

Subjects may discontinue vaccination at any time for any reason or be dropped from vaccination at the discretion of the investigator should any untoward effect occur. In addition, a subject may be discontinued from vaccination by the investigator or the Sponsor if vaccination is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at vaccination discontinuation are provided in Section 7.1.4 – Other Procedures.

A subject must be discontinued from vaccination but continue to be monitored in the trial for any of the following reasons:

- The subject or subject's legally acceptable representative requests to discontinue vaccination.
- The subject's treatment assignment has been unblinded by the investigator, Merck subsidiary or through the emergency unblinding call center and the subject was identified as having been randomized to placebo. (Of note, if a subject's treatment assignment was unblinded by the investigator, Merck subsidiary, or through the emergency unblinding call center, between Dose 1 and Dose 2, and the subject was found to have received active study vaccine, the subject may receive Dose 2 **if not contraindicated**).
- The subject has a medical condition or personal circumstance which, in the opinion of the investigator and/or Sponsor, placed the subject at unnecessary risk from continued administration of study drug/vaccine.
- The subject has a confirmed positive serum pregnancy test, or new intent or desire to become pregnant.
- The subject experiences an allergic reaction to a component of the study vaccine/placebo.

For subjects who are discontinued from vaccination but continue to be monitored in the trial, see Section 6.0, and Section 7.1.5.3 for those procedures to be completed at each specified visit.

Discontinuation from vaccination is "permanent." Once a subject is discontinued, he/she shall not be allowed to restart vaccination.

5.8.2 Withdrawal from the Trial

A subject must be withdrawn from the trial if the subject or subject's legally acceptable representative withdraws consent from the trial.

If a subject withdraws from the trial, they will no longer receive vaccination or be followed at scheduled protocol visits.

Specific details regarding procedures to be performed at the time of withdrawal from the trial including the procedures to be performed should a subject repeatedly fail to return for scheduled visits and/or if the study site is unable to contact the subject, as well as specific details regarding withdrawal from Future Biomedical Research are outlined in Section 7.1.4 – Other Procedures.

5.9 Subject Replacement Strategy

A subject who discontinues from the trial will not be replaced.

5.10 Beginning and End of the Trial

The overall trial begins when the first subject signs the informed consent form. The patient participation portion of the trial ends when the last subject completes the last study-related phone-call or visit, discontinues from the trial or is lost to follow-up (i.e. the subject is unable to be contacted by the investigator). For purposes of analysis and reporting, the overall trial ends when the Sponsor receives the external clinical data point, e.g., serology assay result or patient data from the last study-related phone-call or visit.

5.11 Clinical Criteria for Early Trial Termination

The siDMC will be provided with stopping rules as guidance for temporarily pausing or terminating the study. If any of the following events occurs, administration of study vaccine will be temporarily paused until a thorough review of accumulated safety data is undertaken by the siDMC:

1. Death in any subject, unless the cause of death is due to obvious alternative etiology.
2. Any serious adverse event (SAE) that is deemed to be related to the study vaccine.
3. Unexpected life-threatening adverse events in any subject, unless due to obvious alternative etiology.
4. Two or more subjects in a vaccination group (TV003 or TV005) experience a Grade 3 or greater laboratory abnormality that is related to administered vaccine.

Guidelines for the siDMC are located in the Investigator Trial File Binder.

6.0 TRIAL FLOW CHART

Screening Procedures for All Subjects (-28 days to -1 day)

Time Relative to Dose 1 (-28 days to -1 day)
Screening Procedures
Study informed consent [a]
Informed consent for future biomedical research (FBR) [a]
Distribute Dengue Informational Brochure
Enter subject in Interactive Response Technology
Full physical examination [b]
Medical history [c]
Assessment of Inclusion/Exclusion Criteria
Review prior/concomitant medications and vaccinations
Screening Testing
Urine sample ▪ Urinalysis ▪ Urine drug testing
Blood sample for serology testing ▪ Hematology / chemistry ▪ HIV, Hepatitis B, and Hepatitis C ▪ Pregnancy testing [d] ▪ Alcohol testing (blood or breath testing) ▪ Hemoglobin A1c (HbA1c) [e]

[a] Consent must be obtained PRIOR to any study procedures, including screening.
[b] Full physical examination includes collecting vital signs, body weight and height; assessment of head, eyes, ears, nose and throat; auscultation of the heart and lung; and examination of the abdomen, skin, lymph nodes, neurological system, and musculoskeletal system.
[c] A medical history for the prior 5 years will be obtained, including a history of flavivirus vaccination or natural flavivirus infection at any time.
[d] For all females of childbearing potential, a serum pregnancy test (sensitive to detect beta-human chorionic gonadotropin [β-hCG] at concentrations of 25 IU/L or higher) will be obtained at the screening visit.
[e] For those subjects who report a medical history of diabetes mellitus during screening, and cannot provide an HbA1c level within 3 months prior to screening, perform HbA1c level to assess whether subject is excluded from the study based upon this criterion.

Study Procedures for All Subjects (Day 1 to Year 1 PD2)

Visit or Contact and Time Relative to Dose 1	Visit 1 Day 1 (Dose 1)	Visit 2 7 days PD1	Visit 3 12 days PD1	Contact 21 days PD1	Visit 4 28 days PD1	Visit 5 56 days PD1	Contact Month 3 [a] PD1	Contact Month 4 [a] PD1	Contact Month 5 [a] PD1
Visit window permitted (day) [a]		+2 days	-/+2 days [j]	+2 days	+7 days	+7 days	+/-7 days	+/-7 days	+/-7 days
Study Procedures				Safety follow-up contact			Safety follow-up contact	Safety follow-up contact	Safety follow-up contact
Vital Signs	X	X	X		X	X			
Medical history/New medical history [b]	X	X	X		X	X			
Assessment of Inclusion/Exclusion Criteria	X								
Targeted physical examination [c]	X								
Review of prior/concomitant medications/vaccines	X	X	X		X	X			
Distribute electronic Vaccination Report Card (eVRC) [d]	X								
Distribute Subject Identification Card	X								
Randomization in IRT	X								
Safety Evaluations									
Blood sample for laboratory testing (with viremia tested at Visits 2, 3, and 4) [e]	X	X	X		X				
Urine sample for urinalysis [e]	X	X	X		X				
Pregnancy testing [f]	X								
Review of eVRC [d]		X	X		X				
Review of adverse events [g]	X	X	X		X	X			
Specimen Collection									
Blood (DNA) for Future Biomedical Research [h]	X								
Serum samples for flavivirus testing (Dengue IgG ELISA) [i]	X								
Serum samples for antibody responses (VRNT) [i]	X				X	X			
Serum samples for assay development [i]	X				X				
Administration of Study Vaccine/ Placebo									
Administer study vaccine/placebo	X								
Observe subjects for 30 minutes after each vaccination for immediate adverse events	X								

[a] To calculate subsequent visit windows, assume that 1 month equals 30 days and 1 week equals 7 days.
[b] Including a history of flavivirus vaccination or natural flavivirus infection at any time.
[c] Targeted physical examination includes collecting vital signs, auscultation of the heart and lung, examination of the abdomen and lymph nodes, and other assessments directed by the interval medical history.
[d] An eVRC will be distributed and initiated at Visit 1, and subjects will be trained on its use. The eVRC will be reviewed at 7, 12, and 28 days PD1 (Visits 2, 3, and 4) and 7, 12, and 28 days PD2 (Visits 7, 8, and 9).
[e] All blood and urine should be collected for safety evaluations prior to administration of study vaccine/placebo. The results of the safety evaluations do not have to be available prior to administering the vaccine. Viremia will be tested at 7, 12, and 28 days PD1 (Visits 2, 3, and 4) and 7, 12, and 28 days PD2 (Visits 7, 8, and 9). Blood samples for viremia RT-PCR will be collected at the same time as blood for safety labs, and should be aliquoted into 2 serum tubes, and managed as outlined in footnote i.
[f] For all females of childbearing potential, a urine pregnancy test (sensitive to detect β-hCG at concentrations of 25 IU/L or higher) will be conducted prior to vaccination at Visit 1 (Day 1/Dose 1) and Visit 6 (Month 6 PD1 visit/Dose 2).
[g] Adverse event (serious and non-serious) data will be collected shortly after each vaccination. Adverse events (serious and non-serious) are to be reported 1 to 28 days following each dose, which includes the day of vaccination. Dengue-related adverse events (i.e., laboratory confirmed DF, DHF, or DSS, regardless of seriousness), serious AEs, and deaths due to any cause are to be reported for the time period beginning at informed consent through 1 year following the last vaccination.
[h] Informed consent for future biomedical research (FBR) samples must be obtained to collect the DNA sample.
[i] Serum for the Dengue IgG ELISA, Dengue VRNT, and assay development specimens should be aliquoted into 2 serum tubes. One tube will be shipped to a prespecified central laboratory and 1 tube will remain at the site as a back-up sample in case the main sample is destroyed in any way. The Sponsor will notify the site when the back-up samples can be shipped to the laboratory. Leftover main study serum will be stored for future biomedical research if the subject consents to future biomedical research. Refer to the laboratory manual for guidance regarding sample blood volumes and other laboratory procedures.
[j] Visits 3 and 8 are to be conducted 12 to 14 days PD1 and PD2, respectively, unless a subject is experiencing postvaccination rash. These visits may be conducted 10 to 14 days postvaccination for subjects who experience postvaccination rash. Subjects with postvaccination rash should schedule these visits as soon as possible within this time window.

PD = Postdose; DF = Dengue Fever; DHF = Dengue hemorrhagic fever; DSS = Dengue Shock Syndrome; eVRC = Electronic Vaccination Report Card; VRNT = Virus Reduction Neutralization Test; IRT = Interactive Response Technology; DNA = deoxyribonucleic acid; IgG-ELISA = immunoglobulin-Enzyme-Linked Immunosorbent Assay; RT-PCR = reverse transcriptase-polymerase chain reaction; β-hCG = beta-human chorionic gonadotropin; IU/L = international units per liter; AE = adverse event

Study Procedures for All Subjects (Day 1 to Year 1 PD2) (Cont.)

Visit or Contact and Time Relative to Dose 2	Visit 6 Month 6 PD1/ (Dose 2)	Visit 7 7 days PD2	Visit 8 12 days PD2	Contact 21 days PD2	Visit 9 28 days PD2	Contact Month 3 [a] PD2	Contact Month 4 [a] PD2	Contact Month 5 [a] PD2	Visit 10 Month 6 [a] PD2	Contact Month 7 [a] PD2	Contact Month 8 [a] PD2	Contact Month 9[a] PD2	Contact Month 10 [a] PD2	Contact Month 11 [a] PD2	Visit 11 1 Year PD2
Visit window permitted (day) [a]	+ 7 days from Day 180 PD1	+2 days	+/-2 days [i]	+2 days	+7 days	+/-7 days	+/-7 days	+/-7 days	+/-7 days	+/-7 days	+/-7 days	+/-7 days	+/-7 days	+/-7 days	+/-7 days
Study Procedures				Safety follow-up contact		Safety follow-up contact	Safety follow-up contact	Safety follow-up contact		Safety follow-up contact	Safety follow-up contact	Safety follow-up contact	Safety follow-up contact	Safety follow-up contact	
New medical history [b]	X	X	X		X				X						X
Review prior/concomitant medications/vaccines	X	X	X		X				X						X
Vital signs	X	X	X		X				X						X
Targeted physical examination [c]	X														
Initiate Dose 2 in IRT	X														
Initiate electronic Vaccination Report Card (eVRC) for Dose 2 [d]	X														
Safety Evaluations															
Blood sample for laboratory testing (with viremia tested at Visits 7, 8, and 9) [e]	X	X	X		X										
Urine sample for urinalysis [e]	X	X	X		X										
Pregnancy testing [f]	X														
Review of eVRC [d]		X	X		X										
Review of adverse events [g]	X	X	X		X				X						X
Specimen Collection															
Serum samples for antibody responses (VRNT) [h]	X				X				X						X
Serum samples for assay development [h]	X														
Administration of Study Vaccine/Placebo															
Administer study vaccine/placebo	X														
Observe subjects for 30 minutes postvaccination for immediate adverse events	X														

[a] To calculate subsequent visit windows, assume that 1 month equals 30 days and 1 week equals 7 days. Monthly follow-up contacts will use available technology (e.g., phone call, text message, e-mail).
[b] Including a history of flavivirus vaccination or natural flavivirus infection at any time.
[c] Targeted physical examination includes vital signs, auscultation of the heart and lungs, examination of the abdomen and lymph nodes, and other assessments directed by the interval medical history.
[d] The eVRC will be initiated again at Visit 6 (Month 6 PD1/Dose 2). The eVRC will be reviewed at 7, 12, and 28 days following each dose.
[e] All blood and urine should be collected for safety evaluations prior to administration of study vaccine/placebo. The results of the safety evaluations do not have to be available prior to administering the vaccine. Viremia will be tested at 7, 12, and 28 days PD1 (Visits 2, 3, and 4) and 7, 12, and 28 days PD2 (Visits 7, 8, and 9). Blood samples for viremia RT-PCR will be collected at the same time as blood for safety labs, and should be aliquoted into 2 serum tubes, and managed as outlined in footnote h.
[f] For all females of childbearing potential, a urine pregnancy test (sensitive to detect β-hCG at concentrations of 25 IU/L or higher) will be conducted prior to vaccination at Visit 1 (Day 1/Dose 1) and Visit 6 (Month 6 PD1/Dose 2).
[g] Adverse events (serious and non-serious) will be collected shortly after each vaccination. Adverse events (serious and non-serious) are to be reported 1 to 28 days following each dose, including the day of vaccination. Dengue-related adverse events (i.e., laboratory confirmed DF, DHF, or DSS, regardless of seriousness), serious adverse events, and deaths due to any cause are to be reported for the time period beginning at informed consent through 1 year following the last vaccination.
[h] Serum for the Dengue VRNT and assay development specimens should be aliquoted into 2 serum tubes. One tube will be shipped to a prespecified central laboratory and 1 tube will remain at the site as a back-up sample in case the main sample is destroyed in any way. The Sponsor will notify the site when the back-up samples can be shipped to the laboratory. Leftover main study serum will be stored for future biomedical research if the subject consents to future biomedical research. Refer to the laboratory manual for guidance regarding sample blood volumes and other laboratory procedures.
[i] Visit 3 and Visit 8 are to be conducted 12 to 14 days PD1 and PD2, respectively, unless a subject experiences a postvaccination rash. These visits may be conducted 10 to 14 days postvaccination for subjects who experience postvaccination rash. Subjects with postvaccination rash should schedule these visits as soon as possible within this time window.

PD = Postdose; DF = Dengue Fever; DHF = Dengue hemorrhagic fever; DSS = Dengue Shock Syndrome; eVRC = Electronic Vaccination Report Card; VRNT = Virus Reduction Neutralization Test; RT-PCR = reverse transcriptase-polymerase chain reaction; β-hCG = beta-human chorionic gonadotropin; IU/L = international units per liter; AE = adverse event

Study Procedures Subjects Requiring Evaluation for Suspected Dengue

(Unscheduled Visits, Full Duration of Study)

	Acute Initial Evaluation Visit	Convalescent Follow-up Visit 14 to 28 Days After Acute Visit [f]
Study Procedures		
Clinical evaluation for dengue [a]	X	X
Vital signs	X	X
Targeted physical examination [b]	X	X
New medical history [c]	X	X
Review prior/concomitant medications/vaccines	X	X
Safety Evaluations		
Review of adverse events [d]	X	X
Specimen Collection		
Specimen collection for safety labs (chemistry, hematology, urinalysis)	X	X
Serum sample for zika and chikungunya testing (PCR) [e]	X	
Serum sample for virological confirmation of dengue with qualitative and/or quantitative serotype-specific RT-PCR [e]	X	
Serum sample for dengue antibody test with VRNT [e]		X

[a] For subjects who report fever of ≥38.0°C (≥100.4°F) for at least 2 consecutive days, investigators will conduct a clinical evaluation using 2009 WHO Dengue Guidelines (Protocol Section 12.4).

[b] Targeted physical examination includes vital signs, auscultation of the heart and lungs, examination of the abdomen and lymph nodes, and other assessments directed by the interval medical history.

[c] Includes new medical history of natural flavivirus infection at any time.

[d] For the time period beginning at informed consent through 1 year following the last vaccination, dengue-related adverse events (i.e., laboratory confirmed DF, DHF, DSS, regardless of seriousness), serious adverse events, and deaths due to any cause are to be reported.

[e] The serum for the zika and chikungunya RT-PCR, Dengue RT-PCR, and Dengue VRNT specimens should be aliquoted into 2 serum tubes. One tube will be shipped to a prespecified central laboratory and 1 tube will remain at the site as a back-up sample in case the main sample is destroyed in any way. The Sponsor will notify the site when the back-up samples can be shipped to the laboratory.

[f] If the investigator assesses a case as possible/probable dengue, a subsequent convalescent visit will be scheduled 14 to 28 days after the acute initial evaluation visit to assess if the subject has recovered from the suspected dengue case. Clinical evaluation and serum collection for a dengue antibody test will be conducted. Additional convalescent follow-up visit(s) may be scheduled to follow a dengue case to resolution if clinically indicated

VRNT = Virus Reduction Neutralization Test; PCR = Polymerase chain reaction; RT-PCR = Reverse transcription polymerase chain reaction; DF = Dengue Fever; DHF = Dengue hemorrhagic fever; DSS = Dengue Shock Syndrome

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the investigator and or the Sponsor for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The investigator or qualified designee must obtain documented consent from each potential subject or each subject's legally acceptable representative prior to participating in a clinical trial or Future Biomedical Research. If there are changes to the subject's status during the trial (e.g., health or age of majority requirements), the investigator or qualified designee must ensure the appropriate consent is in place.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

7.1.1.1.2 Consent and Collection of Specimens for Future Biomedical Research

The investigator or qualified designee will explain the Future Biomedical Research consent to the subject, answer all of his/her questions, and obtain written informed consent before performing any procedure related to the Future Biomedical Research sub-trial. A copy of the informed consent will be given to the subject.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Subject Identification Card

All subjects will be given a Subject Identification Card identifying them as participants in a research trial. The card will contain trial site contact information (including direct telephone numbers) to be utilized in the event of an emergency. The investigator or qualified designee will provide the subject with a Subject Identification Card immediately after the subject provides written informed consent. At the time of treatment allocation/randomization, site personnel will add the treatment/randomization number to the Subject Identification Card.

The subject identification card also contains contact information for the emergency unblinding call center so that a health care provider can obtain information about trial medication/vaccination in emergency situations where the investigator is not available.

7.1.1.4 Medical History

A medical history (prior 5 years) will be obtained at the screening visit by the investigator or qualified designee. The medical history will include a history of flavivirus vaccination or natural flavivirus infection at any time. At all subsequent trial visits specified in the Trial Flow Chart (Section 6.0), the investigator or qualified designee will record any condition not already recorded as baseline medical history or adverse events on the update medical history electronic case report form (eCRF).

7.1.1.5 Prior and Concomitant Medications Review

7.1.1.5.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 30 days before receipt of the first dose of the trial vaccination.

7.1.1.5.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. Any concurrent medication or medical treatment must be recorded on the appropriate eCRF.

7.1.1.6 Assignment of Screening Number

All consented subjects will be given a unique screening number that will be used to identify the subject for all procedures that occur prior to randomization or treatment allocation. Each subject will be assigned only one screening number. Screening numbers must not be re-used for different subjects.

Any subject who is screened multiple times will retain the original screening number assigned at the initial screening visit.

Specific details on the screening visit requirements (screening/rescreening) are provided in Section 7.1.5.1.

7.1.1.7 Assignment of Treatment/Randomization Number

All eligible subjects will be randomly allocated and will receive a treatment/randomization number. The treatment/randomization number identifies the subject for all procedures occurring after treatment allocation/randomization. Once a treatment/randomization number is assigned to a subject, it can never be re-assigned to another subject.

A single subject cannot be assigned more than 1 treatment/randomization number.

7.1.1.8 Trial Compliance

7.1.1.8.1 Study Vaccination

Unblinded study personnel not otherwise involved in the conduct of the study will prepare the study vaccine. Unblinded study personnel should not have contact with subjects for any study-related procedures/assessments after administration of study vaccines, which includes all safety follow-up procedures.

All safety and immunogenicity assessments will be conducted by blinded personnel, and the subject will be blinded to the study vaccine. Vaccination information, such as Component Identification Number and time of vaccination, must be recorded on the appropriate eCRF as per the Data Entry Guideline (DEG) instructions.

7.1.1.8.2 Exercise and Diet

Subjects should refrain from unusual, unaccustomed strenuous, vigorous physical exercise/activity (e.g., beginning new weight-lifting, running, or bicycling regimens) for 72 hours prior and 28 days following each vaccination. Subjects should maintain a normal diet unless modifications are required to manage an adverse event such as diarrhea, nausea, or vomiting.

7.1.1.9 Dispense Electronic Vaccination Report Cards

The vaccination report card was developed to be administered electronically via a hand-held device. This item was structured as recommended in the final FDA Patient Reported Outcome (PRO) Guidance. The investigator or delegate will train the subject/legal guardian in the use of the electronic vaccination report card prior to dispensing it at Visit 1.

Body temperatures, injection-site AEs, eVRC-prompted systemic complaints, other complaints or illnesses, and medications will be recorded on the eVRC throughout the study. The investigator or delegate will review the data captured on the eVRC with the subject/legal guardian at Visits as specified in the Trial Flow Chart (Section 6.0).

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Physical Examination

A full or targeted physical examination will be performed at specific study visits as specified in the Trial Flow Chart (Section 6). Findings related to the physical examinations should be documented in the subject chart/source documentation. Any clinically significant abnormality will be recorded on the appropriate eCRF.

A **full physical examination** includes vital signs (heart rate, respiratory rate, seated blood pressure, and oral temperature), body weight and height; assessment of head, eyes, ears, nose and throat; auscultation of the heart and lung; and examination of the abdomen, skin, lymph nodes, neurological system, and musculoskeletal system.

A **targeted physical examination** includes vital signs (heart rate, respiratory rate, seated blood pressure, and oral temperature), auscultation of the heart and lungs, examination of the abdomen and lymph nodes, and other assessments directed by the interval medical history.

At vaccination visits, physical examinations are to be performed prior to vaccination.

7.1.2.2 Vital Signs

Vital signs (heart rate, respiratory rate, seated blood pressure, and oral temperature) will be measured at all study visits as specified in the Trial Flow Chart (Section 6). Abnormal vital signs must be confirmed by repeat testing after 15 minutes. Findings related to the vital signs should be documented in the subject chart/source documentation. Any clinically significant abnormality will be recorded on the appropriate eCRF.

At vaccination visits, vital signs are to be performed prior to vaccination.

7.1.2.3 Administration of Vaccine/Placebo

7.1.2.3.1 Preparation of Study Vaccine/Placebo

As specified in Section 5.2.1.1, the study will require 1 or more unblinded individuals to perform the preparation of the study vaccine/placebo. An unblinded individual(s) will be responsible for field-mixing multiple study vaccine/placebo substances (i.e., each individual attenuated viral component and diluent) in order to produce the final study vaccine/placebo formulation that will be administered to the subject. The unblinded individual(s) will be provided with a Pharmacy Manual with the instructions for preparing the field-mixed formulations for all vaccination doses.

In order to avoid bias, the unblinded individual(s) will not be involved in any postvaccination safety assessment procedures. The unblinded individual(s) also must not disclose any information regarding the allocation of the clinical supplies or the appearance of the study

vaccine/placebo to any blinded member of the site staff. No blinded member of the site staff should have contact with the clinical supplies at any point during the course of the study.

7.1.2.3.2 Administration of Study Vaccine/Placebo

A blinded individual should administer the study vaccine/placebo subcutaneously using the syringe that was prepared by the unblinded individual (e.g., unblinded pharmacist). A separate, sterile syringe and needle or sterile disposable unit should be used for the administration of study vaccine/placebo to each subject to prevent transmission of infectious agents from one person to another. Needles should not be recapped. Safe disposal procedures should be followed. Adequate treatment provision, including epinephrine, should be available for immediate use should an anaphylactic or anaphylactoid reaction occur.

Details of each vaccination should be documented on the appropriate eCRF. Because study vaccine/placebo will be shipped in an open-label manner to the site unblinded individual(s), some data related to dispensing study vaccine/placebo may need to be entered by the unblinded individual(s) in order to maintain the study blinding. Only unblinded individual(s) would have access to these eCRFs.

7.1.2.4 Assessment of Adverse Events (Review of eVRC)

Study subjects will be observed for 30 minutes following each vaccination for any immediate adverse events. If any immediate adverse events are observed during this period, the time at which the event occurred within this timeframe, as well as the event itself, any concomitant medications that were administered, and resolution of the event, must be recorded on the appropriate eCRF.

Subjects will record subsequent events using the eVRC. Distribution of the eVRC will occur at Visit 1 and review of the eVRC will occur at visits specified in the Trial Flow Chart (Section 6.0). Instructions on completing the eVRC will be reviewed with each study subject/legal guardian at visits specified in the Trial Flow Chart (Section 6.0).

Daily oral temperature, as well as injection-site and systemic AEs occurring 1 to 28 days following each vaccination will be recorded by the subject/legal guardian on the eVRC. Oral temperature should be measured with the thermometer provided for the study. If the temperature is measured with another thermometer, a note should be entered in the eVRC. This will not be considered a protocol violation. Electronic Vaccination Report Card-prompted injection-site AEs include injection-site pain, erythema, and swelling from 1 to 5 days following each vaccination. Electronic Vaccination Report Card-prompted systemic AEs comprise rash, fatigue, malaise, headache, and myalgia from 1 to 28 days following each vaccination.

Non-serious and serious adverse events will be graded for toxicity as per Appendix 12.6. The investigator will use the information provided by the subject both on the eVRC, and verbally at the time of eVRC review, to apply the appropriate toxicity grade (1 through 4). The grade assigned by the investigator will be recorded in the electronic database on the corresponding eCRF. All deaths will be assessed as Toxicity Grade 4. An alpha scale (A through E) will be used to measure injection-site reactions of redness and swelling. Appendix 12.6 outlines the conversion from alpha scale to toxicity grade for injection-site

reactions. This toxicity grading is in addition to the grading of adverse events by maximum intensity described in Table 3.

Section 7.2 provides detailed information concerning the assessment and recording of adverse events.

7.1.2.5 Assessment of Rash

Subjects who experience rash during the primary postvaccination safety period (1 to 28 days following each vaccination) will be asked to categorize the rash intensity as mild, moderate, or severe based on the following guidance:

Mild: a few scattered red spots with or without small bumps (mild itching may or may not be present);

Moderate: red spots and/or red patches, with or without small bumps, covering a few parts of the body (mild or moderate itching may be present);

Severe: many and/or large red spots and red patches, with bumps, covering most of the trunk and/or other parts of the body (moderate to severe itching may be present)

The subject's rash description will be recorded on the eVRC. Subjects should seek medical care for possible dengue-related symptoms at any time during the study.

Of note, Visit 3 and Visit 8 are to be conducted from 12 to 14 days PD1 and PD2, respectively, unless a subject experiences a postvaccination rash. These visits may be conducted from 10 to 14 days postvaccination for subjects who experience postvaccination rash. Subjects with a postvaccination rash should schedule these visits as soon as possible within this time-window.

The eVRC report of rash will be reviewed and assessed by the study investigator or delegate at the next study visit.

Guidance will be provided to the subject and study site personnel (in the Investigator Trial File Binder) that rashes associated with acute visit for suspected dengue (see Section 7.1.2.6 for more details) will also be thoroughly documented for the duration of the study, regardless of seriousness.

7.1.2.6 Assessment of Suspected Dengue Disease

For the first 28 days after each vaccination, subjects should measure and record their temperature daily on the eVRC. Outside of the 28-day period following each vaccination, subjects should be advised to take and record their temperature daily only when they feel febrile until the febrile episode resolves.

Subjects who experience temperature of ≥38.0°C (≥100.4°F) for ≥2 consecutive days following any vaccination at any time during the study should contact the site as soon as possible to schedule an evaluation visit for suspected dengue (acute visit). The acute initial evaluation visit should occur as close as possible to the second day of protocol-specified fever, and no later than 3 days after the second day of protocol-specified fever. At the acute visit, the investigator will perform a clinical evaluation to assess the subject for dengue disease symptoms and severity. Serum samples will be obtained from the subject to determine the presence of dengue disease.

If the investigator assesses a case as possible/probable dengue, a subsequent convalescent visit will be scheduled to occur 14 to 28 days after the initial evaluation visit to assess if the subject has recovered from the suspected dengue case. During the convalescent visit, the investigator will conduct a clinical evaluation and obtain serum samples for dengue antibody testing. Guidelines for the investigator's clinical assessment of suspected dengue are located in the Investigator Trial File Binder.

All subjects will be contacted (phone, text message, or email) at time points outlined in Section 6.0 to determine if they have experienced temperature of ≥38.0°C (≥100.4^{0}F) for >2 consecutive days or other dengue-related adverse events, and to remind them to present to the study site for evaluation in the event of >2 consecutive days of protocol-specified fever. Safety contacts will continue through 1 year following the last vaccination.

Subjects should seek medical care for possible dengue-related symptoms at any time during the study.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. The total amount of blood/tissue to be drawn/collected over the course of the trial (from pre-trial to post-trial visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per subject can be found in Section 12.3.

7.1.3.1 Laboratory Safety Evaluations (Hematology, Chemistry, and Urinalysis)

Laboratory tests for hematology, chemistry and urinalysis are specified in Table 2. Standard tests (i.e., those performed at each visit at which a blood sample for safety evaluation is required) are specified in the hematology, chemistry, and urinalysis columns of the table and tests performed only at screening are listed in the 'Other' column in the table. In addition to the testing specified on Table 2, blood samples will be obtained at 7, 12, and 28 days following each vaccination and assessed for vaccine virus viremia using a PCR-based assay.

Table 2 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other (screening only)
Hematocrit	Albumin	Blood	hepatitis B surface antigen (HBsAg)
Hemoglobin	Alkaline phosphatase	Glucose	human immunodeficiency virus (HIV) antibody
Platelet count	Alanine aminotransferase (ALT)	Protein	hepatitis C antibody
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	Estimated glomerular filtration rate (eGRF)
	Bicarbonate	Microscopic exam, if abnormal results are noted	HbA1c level [a]
	Calcium		Urine drug screen
	Chloride		Alcohol testing (blood or breath testing)
	Creatinine		
	Glucose		

Hematology	Chemistry	Urinalysis	Other (screening only)
	Phosphorus		
	Potassium		
	Sodium		
	Total Bilirubin		
	Direct Bilirubin, if total bilirubin is elevated above the upper limit of normal		
	Total protein		
	Blood Urea Nitrogen		

[a] For those subjects who report a medical history of diabetes mellitus during screening, and cannot provide an HbA1c level within 4 months prior to screening, perform HbA1c level to assess whether subject is excluded from the study based upon this criterion.

7.1.3.2 Pregnancy Testing

A serum β-hCG pregnancy test (which must be sensitive to detect β-hCG at concentrations of 25 IU/L or higher) will be performed at a central laboratory for women of childbearing potential during screening. In addition, a urine β-hCG pregnancy test (which must be sensitive to detect β-hCG at concentrations of 25 IU/L or higher) will be performed at the site on each vaccination day. A negative urine β-hCG pregnancy test must be documented on the day of vaccination before the administration of study vaccine/placebo. Additional pregnancy tests may be performed at the discretion of the investigator at any time during the study.

If pregnancy develops after the first vaccination, but before completion of the vaccination regimen, the subject will not receive subsequent vaccine doses but may remain in the study and be followed for safety and immunogenicity. If pregnancy develops after the final vaccination, the subject may remain in the study and be followed for safety and immunogenicity. In addition all subjects who become pregnant should be followed to the outcome of the pregnancy (term, miscarriage, abortion, etc.). All pregnancies must be reported as described in Section 7.2.2 to one of the study personnel listed on the Sponsor Contact Information page (located in the Investigator Trial File Binder) as soon as the situation becomes known.

7.1.3.3 Dengue Serostatus Testing

7.1.3.3.1 Dengue IgG ELISA

Dengue IgG ELISA testing will be conducted at Day 1 (Predose) to assess the subject's previous exposure to wild-type or vaccine-induced antibodies for dengue.

Assessment for flavivirus prior exposure will be performed using the Focus Diagnostics Dengue Virus IgG DxSelectTM ELISA diagnostic assay. The Focus Diagnostics Dengue Virus IgG DxSelectTM assay is a qualitative indirect ELISA intended for the detection of IgG antibodies to dengue virus, types 1, 2, 3, and 4 in human serum. Each antigen-coated well contains equal proportions of inactivated, purified dengue viruses types 1 to 4. Diluted serum samples and controls (IgG detectable control, IgG non-detectable control and IgG cut off calibrator) are incubated in the wells to allow specific antibody present in the samples to react with the antigen. Nonspecific reactants are removed by washing and peroxidase-conjugated anti-human IgG is added and reacts with specific IgG present in the sample.

Excess conjugate is removed by washing. Enzyme substrate is added, and the color is allowed to develop. The resultant color change is quantified by a spectrophotometric reading of optical density (OD) at 450 nm which is directly proportional to the amount of antigen-specific IgG present in the sample. All subject results are reported as index values relative to the Cut-off Calibrator. An index value of >1.00 is presumptive for the presence of IgG antibodies to dengue virus. An index value of <1.00 indicates no IgG antibodies to dengue virus were detected. This test will be used in conjunction with medical history, physical exam, and baseline VRNT results to assess subject flavivirus serostatus.

7.1.3.3.2 Virus Reduction Neutralization Test for DENV1, DENV2, DENV3, and DENV4

The Virus Reduction Neutralization Test (VRNT) for DENV1, DENV2, DENV3, and DENV4 will be conducted at Day 1 (Predose) to assess the subject's previous exposure to wild-type or vaccine-induced antibodies for dengue, and provide a baseline for the subject's dengue antibodies. In addition, this neutralization assay will be conducted at the time points outlined in Section 4.2.3.2 to assess the ability of the V181 formulations (TV003 / TV005) to induce neutralizing antibodies to the 4 dengue vaccine serotypes.

The purpose of this neutralization assay is to measure the concentration of dengue virus-neutralizing antibodies in human serum before and after vaccination. Neutralization assays are the most widely used assay in the field to determine virus-neutralizing antibody titers. While no correlate of protection has been established for dengue, the measurement of induction of virus-neutralizing antibody responses provides a mechanism to assess the immunogenicity of the vaccine using a functional assay that may predict the potential for protection.

This neutralization assay for all 4 dengue serotypes will be qualified and executed at the laboratory designated by the Sponsor. The neutralization assay is performed by a serial dilution of serum samples and positive controls, tested in duplicate, and addition of an equal volume of diluted dengue virus (DENV1, DENV2, DENV3, or DENV4) containing a consistent target amount of virus for each given strain to neutralize. Virus control containing diluted virus without serum is also tested. After an incubation period for virus neutralization, each serum/virus mix and virus control is transferred to a tissue culture plate containing confluent Vero cells and incubated to allow for non-neutralized virus adsorption. An incubation period for a specified period of time occurs to allow for replication of the virus. Plates are fixed, and infection is detected by immunostaining using a specific anti-dengue rabbit antibody and a secondary antibody. The titer of a sample is determined by counting and comparing the number of infectious foci in the presence of the test serum to the virus control. The results are reported as an NT titer, which is the reciprocal of the dilution that reduced the number of infectious foci by a defined % compared to the virus control. Calculations are performed using a 4-parameter nonlinear logistic equation.

7.1.3.4 Viremia RT-PCR Testing

The purpose of this test is to evaluate the percentage of individuals with detectable viremia (measured by PCR) for any serotype at 7, 12, and 28 days following each vaccination.

This assay is currently under development and specific processes and assay parameters will be provided in the clinical study report (CSR). The assays in development are a serotype-specific quantitative RT-PCR and a qualitative RT-PCR. The assays are composed of 2 main steps, extraction of RNA from serum specimens, and amplification of the extracted RNA using fluorescent probe and primers. An RNA internal control is used to monitor the extraction process and to detect RT-PCR inhibition.

7.1.3.5 Laboratory Testing for Suspected Dengue Case

7.1.3.5.1 Acute Initial Evaluation Visit

Subjects who report fever of ≥38.0°C (≥100.4°F) for at least 2 consecutive days, will be evaluated for suspected dengue infection at the acute initial evaluation visit, as outlined in Section 6.0.

7.1.3.5.1.1 RT-PCR for Dengue

A qualitative and/or quantitative serotype-specific RT-PCR assay will be used to provide virological confirmation of dengue for subjects with suspected dengue at the acute initial evaluation visit. A description of the RT-PCR assay can be found in Section 7.1.3.4.

7.1.3.5.1.2 RT-PCR for Chikungunya and Zika

Diagnostic RT-PCR assays may be used for the detection of chikungunya and zika viruses. These assays are based upon the real-time amplification of viral genomic RNA sequences from total nucleic acid extraction of the human serum specimen. Both the chikungunya virus and the zika virus reverse transcriptase RT-PCR assays are appropriate in the early days of symptom onset. Chikungunya virus RNA can be detected during the acute phase of illness ≤8 days after symptom onset. Zika virus RNA may be detected in serum for approximately 7 days after symptom onset.

Alternatively and if available for commercial use, the Trioplex Real-time RT-PCR assay may be used for the qualitative detection and differentiation of RNA from zika virus, dengue virus, and chikungunya virus in human sera in dengue-suspected subjects.

7.1.3.5.2 Convalescent Visit

If the investigator assesses a case as possible/probable dengue, a subsequent convalescent visit will be scheduled to occur 14 to 28 days after the acute initial evaluation visit to assess if the subject has recovered from the suspected dengue case.

7.1.3.5.2.1 VRNT for DENV1, DENV2, DENV3, and DENV4

Clinical evaluation and serum collection for a dengue antibody test (VRNT) will be conducted. The VRNT description is outlined in Section 7.1.3.3.2. Additional convalescent follow-up visit(s) may be scheduled to follow a dengue case to resolution if clinically indicated.

7.1.3.6 Laboratory Testing for Immunogenicity

Blood samples for the assessment of immune responses (antibody responses) will be collected from all subjects at the time points specified in Section 4.2.3.2 and the Trial Flow Chart (Section 6.0). Information regarding processing, storing, shipping, etc. of biological specimens is provided in the Specimen Collection Procedures Manual. This information is included in the Investigator Trial File Binder (or equivalent) or a separate Laboratory Manual (also known as the Lab Manual or Specimen Collection Procedures Manual).

Dengue-neutralizing antibody levels for DENV1, DENV2, DENV3, and DENV4 will be measured for the entire study population at the time points listed in Trial Flow Chart (Section 6.0). During the study, samples collected for flavivirus-neutralizing antibody testing also may be used for protocol-specific assay development, validation, and/or immunobridging.

Assay description is located in Section 7.1.3.3.2.

7.1.3.7 Future Biomedical Research Samples

The following specimens are to be obtained as part of Future Biomedical Research:

- DNA for future research
- Leftover main study serum from antibody responses stored for future research
- Leftover main study serum from flavivirus testing stored for future research
- Leftover main study serum from assay development stored for future research

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

Subjects who discontinue/withdraw from vaccination prior to completion of the vaccination regimen should be encouraged to continue to be followed for all remaining study visits.

When a subject discontinues/withdraws from participation in the trial, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events.

7.1.4.1.1 Withdrawal From Future Biomedical Research

Subjects may withdraw their consent for Future Biomedical Research. Subjects may withdraw consent at any time by contacting the principal investigator for the main trial. If medical records for the main trial are still available, the investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com). Subsequently, the subject's consent for Future Biomedical Research will be withdrawn. A letter will be sent from the Sponsor to the investigator confirming the withdrawal. It is the responsibility of the investigator to inform the subject of completion of withdrawal. Any analyses in progress at the time of request for withdrawal or already performed prior to the request being received

by the Sponsor will continue to be used as part of the overall research trial data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main trial are no longer available (e.g., if the investigator is no longer required by regulatory authorities to retain the main trial records) or the specimens have been completely anonymized, there will no longer be a link between the subject's personal information and their specimens. In this situation, the request for specimen withdrawal cannot be processed.

7.1.4.2 Subject Blinding/Unblinding

When the investigator or delegate needs to identify the drug used by a subject and the dosage administered in case of emergency e.g., the occurrence of serious adverse experiences, he/she will contact the emergency unblinding call center by telephone and make a request for emergency unblinding. As requested by the investigator or delegate the emergency unblinding call center will provide the information to him/her promptly and report unblinding to the sponsor. Prior to contacting the emergency unblinding call center to request unblinding of a subject's treatment assignment, the investigator or delegate must enter the intensity/toxicity grade of the adverse experiences observed, the relation to study drug, the reason thereof, etc., in the medical chart etc. Subjects whose treatment assignment has been unblinded by the investigator/delegate and/or non-study treating physician must be discontinued from study drug, but should continue to be monitored in the trial.

Additionally, the investigator must go into the IRT system and perform the unblind in the IRT system to update drug disposition. In the event that the emergency unblinding call center is not available for a given site in this trial, IRT should be used for emergency unblinding in the event that this is required for subject safety.

In the event that unblinding has occurred, the circumstances around the unblinding (e.g., date, reason and person performing the unblinding) must be documented promptly, and the Sponsor Clinical Director notified as soon as possible. Only the principal investigator or delegate and the respective subject's code should be unblinded. Other trial site personnel and Sponsor personnel directly associated with the conduct of the trial should not be unblinded. Subjects whose treatment assignment has been unblinded by the investigator/delegate and/or non-study treating physician must be discontinued from study drug, but should continue to be monitored in the trial.

If the subject's treatment assignment is unblinded by the investigator, Merck subsidiary or through the emergency unblinding call center, after Dose 1 but before receiving Dose 2, separate actions must be taken based on which vaccination group the subject was assigned.

If the subject was unblinded and had received placebo, the subject should not receive Dose 2 and may continue to be monitored in the trial.

If the subject was unblinded and had received active study vaccine, the subject may either be (1) discontinued from further vaccination if there are any clinical concerns about receiving Dose 2 (subject will continue to be monitored in the study) or (2) the subject may receive Dose 2 if not contraindicated.

7.1.4.3 Calibration of Critical Equipment

The investigator or qualified designee has the responsibility to ensure that any critical device or instrument used for a clinical evaluation/test during a clinical trial that provides important information about inclusion/exclusion criteria and/or safety or efficacy parameters shall be suitably calibrated and maintained to ensure that the data obtained is reliable and/or reproducible. Documentation of equipment calibration must be retained as source documentation at the trial site.

Critical Equipment for this trial includes:

- Refrigerator for storage of placebo/diluent
- Two separate freezers (-60° to -80°C/-76° to -112°F) for storage of vaccine clinical supplies separate from clinical serum samples
- Refrigerated centrifuge.

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.5.1 Screening Procedures

Up to 28 days prior to treatment allocation/randomization, potential subjects will be evaluated to determine that they fulfill the entry requirements as set forth in Section 5.1.

Each subject will be screened after consent is signed, and prior to randomization into the study. The consent form must be signed, and all screening activities completed, within 28 days before Visit 1. The screening procedures are outlined in the Trial Flow Chart (Section 6.0).

The goal of the screening activities is to ensure that the subject is appropriate for vaccination, based on the inclusion and exclusion criteria specified in Sections 5.1.2 and 5.1.3. Each subject will be asked to provide assurance that he/she understands and can comply with all study visits and procedures, including the safety follow-up activities through 1 year following the last vaccination (end of study).

Screening procedures are outlined in Section 6.0. If a subject passes screening but cannot be enrolled within the 28-day screening period, the subject may be rescreened; however, subjects who fail to meet the enrollment criteria are defined as failing screening. These subjects may not be rescreened.

Section 7.1.5.1.1 provides guidelines on how to handle subjects who fail 1 or more of the screening laboratory tests that are not specified in the inclusion/exclusion criteria. These guidelines specify if re-screening is permitted and, if so, provide criteria by which the investigator will determine if these subjects may be enrolled in the study. The Sponsor should be contacted regarding any questions.

7.1.5.1.1 Guidelines for Determining if Screening May be Repeated

- If the subject fails to meet the enrollment criteria, the subject fails screening, without possibility for retesting (with the exception of the items marked with an asterisk in Section 5.1.3).

- If the subject meets all of the enrollment criteria, but the results of 1 or more of the laboratory tests not specified in the enrollment criteria are outside the normal range, the following choices are available:

  1. The subject may be excluded from the study.
  2. The abnormal test(s) may be repeated.
     a. If the repeat test value(s) are within the normal range, the subject may enter the study.
     b. If the repeat test value(s) are still abnormal, the study investigator will evaluate the findings of the history, prior/concomitant medication(s)/treatment(s) and complete physical examination (obtained as described in Section 7.1.2.1), looking especially for diseases that could result in the abnormal laboratory value in question. If such diseases can be ruled out, and if the abnormal laboratory value is not clinically significant (NCS), then the subject may enter the study. In this case, the investigator must annotate the laboratory value as "NCS" on the laboratory safety test source document).

7.1.5.2 Treatment Period/Vaccination Visit

7.1.5.2.1 Unblinded Preparation of Vaccine

As outlined in Section 5.2.3, the study will require 1 or more unblinded individuals to prepare the study vaccine/placebo. An unblinded individual(s) will be responsible for field-mixing multiple study vaccine/placebo substances (i.e., each individual attenuated viral component and diluent) in order to produce the final study vaccine/placebo formulation that will be administered to the subject. The unblinded individual(s) will be provided with a Pharmacy Manual with the instructions for preparing the field-mixed formulations for all vaccination doses.

In order to avoid bias, the unblinded individual(s) will not be involved in any postvaccination safety assessment procedures. The unblinded individual(s) also must not disclose any information regarding the allocation of the clinical supplies or the appearance of the study vaccine/placebo to any blinded member of the site staff. No blinded member of the site staff should have contact with the clinical supplies at any point during the course of the study.

7.1.5.3 Discontinued Subjects Continuing to be Monitored in the Trial

Subjects who discontinue trial vaccination prior to Dose 2, but agree to continue in the study, will continue to participate in the trial as specified in Section 6.0 - Trial Flow Chart. However, these subjects will not receive Dose 2 at the Month 6 PD1 visit, they will not

receive the eVRC at Month 6 PD1, and there will not be an eVRC to review at the visit at 28 days PD2 visit.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Sponsor's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Sponsor's product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by the Sponsor for human use.

Adverse events may occur during clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

All adverse events that occur after the consent form is signed but before allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure. From the time of allocation/randomization through 28 days following the first vaccination(s) and from the time of any subsequent vaccination(s) through 28 days thereafter, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Electronic reporting procedures can be found in the Electronic Data Capture (EDC) data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor

Administration of 1 or more doses of study vaccine/placebo outside of the 2-dose schedule will be considered an overdose for this protocol, regardless if it results in an adverse event or not.

If an adverse event(s) is associated with ("results from") the overdose of Sponsor's product or vaccine, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Sponsor's product or vaccine meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported by the investigator within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

7.2.2 Reporting of Pregnancy and Lactation to the Sponsor

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure. Pregnancies and lactations that occur from the time of treatment allocation/randomization through the end of the study must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

7.2.3 Immediate Reporting of Adverse Events to the Sponsor

7.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Sponsor's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is an other important medical event.

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Sponsor in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by the Sponsor for collection purposes.

- Is a cancer;
- Is associated with an overdose.

Refer to Table 3 for additional details regarding each of the above criteria.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause, that occurs to any subject must be reported within 24 hours to the Sponsor if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 1 year following the last vaccination, any serious adverse event, or follow up to a serious adverse event, including death due to any cause, whether or not related to the Sponsor's product, must be reported within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Additionally, any serious adverse event brought to the attention of an investigator who is a qualified physician at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor if the event is either:

1. A death that occurs prior to the subject completing the trial, but outside the time period specified in the previous paragraph.

or

2. A serious adverse event that is considered by an investigator who is a qualified physician to be vaccine related.

All subjects with serious adverse events must be followed up for outcome.

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported to the Sponsor.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 14 days following cessation of treatment, any ECI, or follow up to an ECI, whether or not related to the Sponsor's product, must be reported within 24 hours to the Sponsor, either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry

guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Events of clinical interest for this trial include:

1. an overdose of Sponsor's product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

   *Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events with respect to the elements outlined in Table 3. The investigator's assessment of causality is required for each adverse event. Refer to Table 3 for instructions in evaluating adverse events.

Table 3 Evaluating Adverse Events

Maximum Intensity	**Mild**	awareness of sign or symptom, but easily tolerated (for pediatric trials, awareness of symptom, but easily tolerated)
	Moderate	discomfort enough to cause interference with usual activity (for pediatric trials, definitely acting like something is wrong)
	Severe	incapacitating with inability to work or do usual activity (for pediatric trials, extremely distressed or unable to do usual activities) **Injection site redness or swelling from the day of vaccination through Day 5 post-vaccination will be evaluated by maximum size.**
Seriousness	A serious adverse event (AE) is any adverse event occurring at any dose that:	
	†**Results in death; or**	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred [Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.]; or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis); or	
	Is a cancer (although not serious per ICH definition, is reportable to the Sponsor within 24 hours to meet certain local requirements; **or**	
	Is associated with an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the test vaccine to be discontinued?	
Relationship to test vaccine	Did the test vaccine cause the adverse event? The determination of the likelihood that the test vaccine caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test vaccine and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the test vaccine and the AE;** the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the test vaccine caused the adverse event:	
	Exposure	Is there evidence that the subject was actually exposed to the test vaccine such as: reliable history, acceptable compliance assessment (e.g., diary), seroconversion or identification of vaccine virus in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the test vaccine? Is the time of onset of the AE compatible with a vaccine-induced effect?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to test vaccine (continued)	**The following components are to be used to assess the relationship between the test vaccine and the AE: (continued)**	
	Dechallenge	(not applicable for vaccines)
	Rechallenge	Was the subject reexposed to the test vaccine in this trial? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose vaccine trial.) NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE TEST VACCINE, OR IF REEXPOSURE TO THE TEST VACCINE POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR CLINICAL DIRECTOR AND THE INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE.
	Consistency with Trial Vaccine Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the test vaccine or vaccine class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following:	**Use the following criteria as guidance (not all criteria must be present to be indicative of a vaccine relationship).**	
Yes, there is a reasonable possibility of vaccine relationship.	There is evidence of exposure to the test vaccine. The temporal sequence of the AE onset relative to the administration of the test vaccine is reasonable. The AE is more likely explained by the test vaccine than by another cause.	
No, there is not a reasonable possibility of vaccine relationship	Subject did not receive the test vaccine OR temporal sequence of the AE onset relative to administration of the test vaccine is not reasonable OR the AE is more likely explained by another cause than the Sponsor's product. (Also entered for a subject with overdose without an associated AE.)	

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations, i.e., per ICH Topic E6 (R1) Guidelines for Good Clinical Practice.

7.3 TRIAL GOVERNANCE AND OVERSIGHT

7.3.1 Data Monitoring Committee

To supplement the routine monitoring outlined in this protocol, a separate Standing Internal Data Monitoring Committee (siDMC) will monitor the interim data from this trial up to the release of the IA to the Merck headquarters personnel. The siDMC comprises members of Sponsor Senior Management, none of whom are directly associated with the conduct of this trial. The siDMC will monitor the trial at an appropriate frequency (see Section 8.7 - Interim Analyses) for evidence of adverse effects of trial vaccination, up to the release of the IA, as described in the siDMC Charter. The siDMC will determine whether the trial should continue (or other modifications, pre-specified or otherwise, should be made) according to the protocol, considering the overall risk and benefit to trial participants. The siDMC will also make recommendations to the Sponsor protocol team regarding steps to ensure both subject safety and the continued ethical integrity of the trial.

Specific details regarding responsibilities of the siDMC will be described in a separate charter that is reviewed and approved by the siDMC.

8.0 STATISTICAL ANALYSIS PLAN

This section outlines the statistical analysis strategy and procedures for the study. Changes to analyses made after the protocol has been finalized, but prior to unblinding, will be documented in a supplemental SAP (sSAP) and referenced in the CSR for the study. Post hoc exploratory analyses will be clearly identified in the CSR.

8.1 Statistical Analysis Plan Summary

Key elements of the statistical analysis plan are summarized below; the comprehensive plan is provided in Sections 8.2 through 8.12.

Study Design Overview	A Phase 1 Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Immunogenicity of a Live-Attenuated Tetravalent Dengue Vaccine (V181) in Flavivirus-Naïve and Flavivirus-Experienced Healthy Adults
Treatment Assignment	There are 3 vaccination arms. Subjects will be allocated between the continental United States and Puerto Rico and assigned randomly in a 2:2:1 ratio to receive TV003, TV005, or placebo using an IRT. A double-blind/masking technique will be used.
Analysis Populations	Safety: All Subjects as Treated (ASaT) Immunogenicity: Per-Protocol Population

Primary and Secondary Endpoints	Safety (Primary): Serious and non-serious solicited and unsolicited adverse events. Immunogenicity (Secondary): The percentage of subjects seropositive at 28 days PD1. Seropositivity is defined as having VRNT above the lower limit of detection of the assay (titers ≥10).
Statistical Methods for Key Immunogenicity Analysis	Summary by formulation for each serotype. Confidence intervals based on the exact binomial method proposed by Clopper and Pearson.
Statistical Methods for Key Safety Analyses	Variables of key interest (Tier-1 events) are eVRC-prompted injection-site adverse events: pain, erythema, and swelling (1 to 5 days following each vaccination), elevated temperature (≥38.0ºC [≥100.4°F] oral or equivalent), and eVRC-prompted systemic adverse events of rash (1 to 28 days following each vaccination) assessed as toxicity grade 3 or 4 are considered Tier 1 events. P-values and 95% confidence intervals for between-treatment differences in the percentage of subjects with events will be calculated using the Miettinen and Nurminen method [30].
Interim Analysis	An interim analysis to evaluate safety and immunogenicity will be performed at 28 days PD1 when immunogenicity data are available. Protocol violators will be assessed for this analysis.
Multiplicity	No multiplicity adjustment is planned for this Phase 1 study.
Sample Size and Power	Safety: If no vaccine-related serious adverse events are observed among the 80 subjects in each V181 vaccination group, this study will provide 97.5% confidence that the underlying percentage of subjects with vaccine-related serious adverse events is <4.6% in each V181 vaccination group. Immunogenicity: Approximately 10% of subjects are expected to be non-evaluable. Given this rate, we expect approximately 72 evaluable subjects in the V181 vaccination groups. Assuming a seroconversion rate of approximately 90%, the width of the confidence interval is approximately 15 percentage points for summaries involving 72 subjects.

8.2 Responsibility for Analyses/In-House Blinding

The statistical analysis of the data obtained from this study will be the responsibility of the Clinical Biostatistics department of the Sponsor.

This study will be conducted as triple-blind until the release of the IA, and will change to a double-blind study from that point to the end of the study (site personnel and subjects remain blinded). The official, final database will not be unblinded until medical/scientific review has been performed, protocol deviations have been identified, and data have been declared final and complete.

The Clinical Biostatistics department will generate the randomized allocation schedule(s) for study treatment assignment. Randomization will be implemented in an IRT.

For this Phase 1 study, an internal statistician and statistical programmer not directly involved with the conduct of the study will be unblinded from the initiation of the study to perform the planned interim analysis (Section 8.7) and facilitate the interim reviews of the safety data by the siDMC as outlined in the siDMC Charter. After the release of the IA, Merck HQ personnel involved in the conduct of the trial will be unblinded. As such, the

internal statistician and statistical programmer not directly involved with the conduct of the study will not be required after the IA.

All external personnel will remain blinded to the individual vaccination group assignments until the final analysis is conducted at Year 1 PD2.

8.3 Estimation

Objectives of the study are stated in Section 3.0.

8.4 Analysis Endpoints

Immunogenicity and safety endpoints that will be evaluated are listed below.

8.4.1 Immunogenicity Endpoints

The secondary endpoint, which focuses on immunogenicity for each dengue serotype, will be the percentage of subjects seropositive at 28 days PD1. Seropositivity is defined as having VRNT titers above the lower limit of detection of the assay (titers ≥10).

Exploratory endpoints are listed below:

1. GMT of virus-neutralizing antibodies, as measured by VRNT titer separately for each serotype at each immunogenicity assessment time point.
2. GMFR of virus-neutralizing antibodies measured by VRNT separately for each serotype at 28 days post each vaccination compared to prevaccination (Study Day 1 or Study Month 6 for Dose 1 and Dose 2 respectively).
3. The percentage of individuals who are seropositive by VRNT titers ≥10, separately for each serotype, at each immunogenicity assessment time point other than 28 days PD1. Seropositivity is defined as having VRNT above the lower limit of detection of the assay (titers ≥10).
4. The percentage of subjects with detectable viremia (measured by PCR) for any serotype at 7, 12, and 28 days following each vaccination.

8.4.2 Safety Endpoints

An initial description of safety measures is provided in Section 4.2.3.1.

The overall safety and tolerability profile for each formulation will be assessed by clinical review of all safety data collected. Safety measures to be summarized include proportions of subjects with (1) any adverse events, (2) any injection-site adverse events, (3) any systemic adverse events, (4) any vaccine-related systemic adverse events, (5) any serious adverse events, (6) any vaccine-related serious adverse events, (7) any dengue-related adverse events (as defined in Section 2.1); (8) rash (as defined in Section 7.1.2, (9) vaccine virus viremia (as defined in Section 4.2.3.1), and (10) death. Solicited and unsolicited injection-site and systemic adverse events will be summarized overall, and by maximum reported intensity. The oral temperatures and rashes will also be summarized. For this study, temperatures ≥38.0°C (≥100.4°F) oral or equivalent will be considered a fever.

8.5 Analysis Populations

8.5.1 Immunogenicity Analysis Populations

The Per-Protocol population will serve as the primary population for the analysis of the immunogenicity data in this study. The Per-Protocol population excludes subjects due to major deviations from the protocol that may substantially affect the results of the primary immunogenicity endpoints. The key immunogenicity analyses will also be performed using the Full Analysis Set (FAS) population, which consists of all randomized subjects who receive study vaccination and have at least 1 valid serology result. The final determination on protocol violations will be made prior to the final unblinding of the database and will be documented in a separate memo. Subjects will be included in the vaccination group to which they are vaccinated for the analysis of immunogenicity data using both the Per-Protocol and FAS populations. No adjustment will be made for missing data.

8.5.2 Safety Analysis Populations

The All Subjects as Treated (ASaT) population will be used for the analysis of safety data in this study. The ASaT population consists of all randomized subjects who received at least 1 dose of study vaccine. Subjects will be included in the vaccination group corresponding to the study vaccine they actually received for the analysis of safety data using the ASaT population. For most subjects this will be the vaccination group to which they are randomized. Subjects who receive incorrect study vaccine for the entire treatment period will be included in the vaccination group corresponding to the study vaccine actually received. No adjustment will be made for missing data.

8.6 Statistical Methods

This section describes the statistical methods that address the primary and secondary objectives.

The key immunogenicity analyses to be performed are summarized in Table 4 (Section 8.6.1). These analyses will be descriptive with no formal hypothesis testing.

The safety analyses are summarized in Section 8.6.2.

8.6.1 Statistical Methods for Immunogenicity Analyses

The secondary analysis of immunogenicity will be descriptive with no formal hypothesis testing.

Table 4 Summary of Secondary Immunogenicity Analyses

Secondary Analysis/Endpoint	Statistical Method	Analysis Populations	Missing Data Approach
The percentage of subjects seropositive at 28 days PD1. Seropositivity is defined as having VRNT titers above the lower limit of detection of the assay (titers ≥10).	Summary by formulation for each serotype. Confidence intervals on method proposed by Clopper and Pearson.	• Per-Protocol population • Full Analysis Set	Observed data only

8.6.2 Statistical Methods for Safety Analyses

Safety and tolerability will be assessed by clinical review of all relevant parameters.

The analysis of safety results will follow a tiered approach (Table 5). The tiers differ with respect to the analyses that will be performed. Safety parameters or adverse events of special interest that are identified a priori constitute "Tier 1" safety endpoints that will be subject to inferential testing for statistical significance with p-values and 95% confidence intervals provided for between-group comparisons. Other safety parameters will be considered Tier 2 or Tier 3. Tier 2 parameters will be assessed via point estimates with 95% confidence intervals provided for between-group comparisons; only point estimates by treatment group are provided for Tier 3 safety parameters.

Adverse events (specific terms as well as system organ class terms) that are not pre-specified as Tier-1 endpoints will be classified as belonging to "Tier 2" or "Tier 3", based on the number of events observed. Membership in Tier 2 requires that at least 4 subjects in any treatment group exhibit the event; all other adverse events will belong to Tier 3.

The threshold of at least 4 events was chosen because the 95% confidence interval for the between-group difference in percent incidence will always include zero when treatment groups of equal size each have less than 4 events and thus would add little to the interpretation of potentially meaningful differences. Because many 95% confidence intervals may be provided without adjustment for multiplicity, the confidence intervals should be regarded as a helpful descriptive measure to be used in review, not a formal method for assessing the statistical significance of the between-group differences in adverse events and predefined limits of change.

For this protocol, eVRC-prompted injection-site adverse events: pain, erythema, and swelling (1 to 5 days following each vaccination), elevated temperature (≥38.0ºC [≥100.4°F] oral or equivalent), and eVRC-prompted systemic adverse events of rash (1 to 28 days following each vaccination) assessed as toxicity Grade 3 or 4 are considered Tier 1 events. In addition, the broad clinical categories consisting of the percentage of subjects with any AE, a vaccine-related AE, a serious AE, an AE which is both vaccine-related and serious, and who discontinued due to an AE will be considered Tier 2 endpoints. P-values (Tier 1 only) and 95% confidence intervals (Tier 1 and Tier 2) will be provided for between-treatment differences in the percentage of subjects with events; these analyses will be performed using the Miettinen and Nurminen method [30], an unconditional, asymptotic method. Both TV003 and TV005 will be compared to placebo separately for Tier 1 and Tier 2 events.

Table 5 Analysis Strategy for Safety Parameters

Safety Tier	Safety Endpoint	p-value	95% CI for Treatment Comparison	Descriptive Statistics
Tier 1	eVRC-prompted injection-site adverse events: pain, erythema, and swelling	X	X	X
	Elevated temperature (≥38.0ºC [≥100.4°] oral or equivalent)	X	X	X
	eVRC-prompted systemic AEs of rash assessed at toxicity Grade 3 or 4	X	X	X
Tier 2	Any serious AE		X	X
	Any vaccine-related AE		X	X
	Any serious and vaccine-related AE		X	X
	Discontinuation due to AE		X	X
	Specific AEs or SOCs (incidence ≥4 of subjects in 1 of the vaccination groups)		X	X
Tier 3	Specific AEs or SOCs (incidence <4 of subjects in all of the vaccination groups)			X

AE = Adverse Event; SOC = System Organ Class; X = results will be provided.

8.6.3 Summaries of Baseline Characteristics, Demographics, and other Analyses

The comparability of the vaccination groups for each relevant characteristic will be assessed by the use of tables and/or graphs. No statistical hypothesis tests will be performed on these characteristics. The number and percentage of subjects screened and randomized, the primary reasons for screening failure, and the primary reason for discontinuation will be displayed. Demographic variables (e.g., age), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized by vaccination group either by descriptive statistics or categorical tables.

8.7 Interim Analyses

An interim analysis to evaluate safety and immunogenicity will be performed at 28 days PD1 when all data are available (i.e., when ~100% of the safety and immunogenicity data at 28 days PD1 are available) to support internal decision making. An internal statistician and statistical programmer not assigned to the protocol will be unblinded to perform the interim analysis. The summary will display the aggregate immunogenicity and safety data for each vaccination group. The HQ study team will become unblinded at the release of the IA and will perform review of the IA data. An interim determination of protocol violators will be assessed for this analysis.

8.8 Multiplicity

No multiplicity adjustment is planned in this Phase 1 study.

8.9 Sample Size and Power Calculations

8.9.1 Immunogenicity Analysis

This is an estimation study in which 200 subjects will be allocated between the continental United States and Puerto Rico, and randomized in a 2:2:1 ratio to receive TV003, TV005, or placebo using an IRT. Approximately 10% of subjects are expected to be non-evaluable at 28 days PD1. Given this rate, we expect approximately 72 evaluable subjects in the V181 vaccination groups. The precision of the study can be estimated based on hypothetical observed seroconversion rates by consideration of the width of the associated confidence interval. Table 6 presents the associated confidence intervals for hypothetical observed seroconversion rates ranging from 75% to 95.8% for a sample size of 72. The calculation is based on the exact binomial method proposed by Clopper and Pearson (1934) [31]. Assuming a seroconversion rate of approximately 90%, the width of the confidence interval is approximately 15 percentage points for summaries involving 72 subjects.

Table 6 Hypothetical Observed Seroconversion Rates and Associated 95% Confidence Intervals for Evaluable Sample Sizes of 72

Evaluable Sample Size	Hypothetical Observed Seroconversion Rate	95% Confidence Interval
72	75.0% (54/72)	(63.4%, 84.5%)
	80.6% (58/72)	(69.5%, 88.9%)
	84.7% (61/72)	(74.3%, 92.1%)
	90.3% (65/72)	(81.0%, 96.0%)
	95.8% (69/72)	(88.3%, 99.1%)

8.9.2 Safety Analysis

The probability of observing at least 1 vaccine-related serious adverse event in this study depends on the number of subjects vaccinated and the underlying percentage of subjects with a vaccine-related serious adverse event in the study population. If the underlying incidence of a vaccine-related serious adverse event is 0.86% (1 of every 116 subjects receiving the vaccine), there is a 50% chance of observing at least 1 vaccine-related serious adverse event among 80 subjects in each V181 vaccination group. If the incidence rate is 1 of every 51 recipients (1.99%), there is an 80% chance of observing at least 1 vaccine-related serious adverse event. If no vaccine-related SAEs are observed among the 80 subjects in each V181 vaccination group, this study will provide 97.5% confidence that the underlying percentage of subjects with vaccine-related serious adverse event is <4.6% in each V181 vaccination group.

8.10 Subgroup Analyses

The subgroup analyses will be comprised of descriptive summary statistics by baseline serostatus (flavivirus-naïve and flavivirus-experienced) for both immunogenicity and safety profiles.

For the purposes of the subgroup analyses, a subject is considered flavivirus-experienced based on fulfilling any 1 of the following 4 criteria at Day 1 (Predose 1):

- History of flavivirus vaccination at any time
- History of natural flavivirus infection at any time
- Positive dengue IgG ELISA result
- Positive VRNT titer.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by the Sponsor as summarized in Table 7.

Table 7 Product Descriptions

Product Name & Potency	Dosage Form	Source/ Additional Information
V181 rDENV1Δ30 (a dengue 1 vaccine) titer ≥4.0E+03 pfu/mL	1.0 mL sterile solution in a vial	Provided centrally by the Sponsor. Individual strain will be combined to form a field mix as per detailed instructions in the Pharmacy Manual
V181 rDENV2/4Δ30(ME) (a dengue 2 vaccine; low dose), titer ≥4.0E+03 pfu/mL	1.0 mL sterile solution in a vial	Provided centrally by the Sponsor. Individual strain will be combined to form a field mix as per detailed instructions in the Pharmacy Manual
V181 rDENV2/4Δ30(ME) (a dengue 2 vaccine; high dose), titer ≥4.0E+04 pfu/mL	1.0 mL sterile solution in a vial	Provided centrally by the Sponsor. Individual strain will be combined to form a field mix as per detailed instructions in the Pharmacy Manual
V181 rDENV3Δ30/31, (a dengue 3 vaccine) titer ≥4.0E+03 pfu/mL	1.0 mL sterile solution in a vial	Provided centrally by the Sponsor. Individual strain will be combined to form a field mix as per detailed instructions in the Pharmacy Manual

Product Name & Potency	Dosage Form	Source/ Additional Information
V181 rDENV4Δ30 (a dengue 4 vaccine), titer ≥4.0E+03 pfu/mL	1.0 mL sterile solution in a vial	Provided centrally by the Sponsor. Individual strain will be combined to form a field mix as per detailed instructions in the Pharmacy Manual
Leibovitz L-15 medium, placebo	1.2 mL sterile solution in a vial	Provided centrally by the Sponsor. To be used individually, as placebo, and as diluent for field mix preparation as per instructions in the Pharmacy Manual

All placebos were created by the Sponsor to match the active product.

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

Open-label, single-dose vials will be supplied to the unblinded individual(s) at the Clinical site. Supplies will be affixed with a clinical label in accordance with regulatory requirements. Preparation of the field mix will occur by unblinded individuals as per the instructions in the Pharmacy Manual supplied by the Sponsor.

In addition, 2.0-mL sterile empty vials will be provided for on-site dosage preparation.

9.3 Clinical Supplies Disclosure

This trial is blinded but supplies are provided open label; therefore, an unblinded pharmacist or qualified trial site personnel will be used to blind supplies. Vaccine identity (name, strength or potency) is included in the label text; random code/disclosure envelopes or lists are not provided.

The emergency unblinding call center will use the treatment/randomization schedule for the trial to unblind subjects and to unmask vaccine identity. In the event that the emergency unblinding call center is not available for a given site in this trial, the central electronic treatment allocation/randomization system (IRT) should be used in order to unblind subjects and to unmask treatment/vaccine identity. The Sponsor will not provide random code/disclosure envelopes or lists with the clinical supplies.

Vaccine identification information is to be unmasked ONLY if necessary for the welfare of the subject. Every effort should be made not to unblind the subject unless necessary.

In the event that unblinding has occurred, the circumstances around the unblinding (e.g., date, reason and person performing the unblinding) must be documented promptly, and the Sponsor Clinical Director notified as soon as possible. Only the principal investigator or delegate and the respective subject's code should be unblinded. Trial site personnel and Sponsor personnel directly associated with the conduct of the trial should not be unblinded to treatment assignment. Subjects whose treatment assignment has been unblinded (by the

investigator, Merck subsidiary, or through the emergency unblinding call center) must be discontinued from study drug, but should continue to be monitored in the trial.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

9.5 Discard/Destruction/Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from the Sponsor or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial. For all trial sites, the local country Sponsor personnel or designee will provide appropriate documentation that must be completed for drug accountability and return, or local discard and destruction if appropriate. Where local discard and destruction is appropriate, the investigator is responsible for ensuring that a local discard/destruction procedure is documented.

9.6 Standard Policies

Trial site personnel will have access to a central electronic treatment allocation/randomization system (IVRS/IWRS system) to allocate subjects, to assign vaccine to subjects and to manage the distribution of clinical supplies. Each person accessing the IVRS system must be assigned an individual unique PIN. They must use only their assigned PIN to access the system, and they must not share their assigned PIN with anyone.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

10.1.1 Confidentiality of Data

By signing this protocol, the investigator affirms to the Sponsor that information furnished to the investigator by the Sponsor will be maintained in confidence, and such information will be divulged to the institutional review board, ethics review committee (IRB/ERC) or similar or expert committee; affiliated institution and employees, only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees. Data generated by this trial will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

10.1.2 Confidentiality of Subject Records

By signing this protocol, the investigator agrees that the Sponsor (or Sponsor representative), IRB/ERC, or regulatory authority representatives may consult and/or copy trial documents in order to verify worksheet/case report form data. By signing the consent form, the subject agrees to this process. If trial documents will be photocopied during the process of verifying

worksheet/case report form information, the subject will be identified by unique code only; full names/initials will be masked prior to transmission to the Sponsor.

By signing this protocol, the investigator agrees to treat all subject data used and disclosed in connection with this trial in accordance with all applicable privacy laws, rules and regulations.

10.1.3 Confidentiality of Investigator Information

By signing this protocol, the investigator recognizes that certain personal identifying information with respect to the investigator, and all subinvestigators and trial site personnel, may be used and disclosed for trial management purposes, as part of a regulatory submissions, and as required by law. This information may include:

1. name, address, telephone number and e-mail address;
2. hospital or clinic address and telephone number;
3. curriculum vitae or other summary of qualifications and credentials; and
4. other professional documentation.

Consistent with the purposes described above, this information may be transmitted to the Sponsor, and subsidiaries, affiliates and agents of the Sponsor, in your country and other countries, including countries that do not have laws protecting such information. Additionally, the investigator's name and business contact information may be included when reporting certain serious adverse events to regulatory authorities or to other investigators. By signing this protocol, the investigator expressly consents to these uses and disclosures.

If this is a multicenter trial, in order to facilitate contact between investigators, the Sponsor may share an investigator's name and contact information with other participating investigators upon request.

10.1.4 Confidentiality of IRB/IEC Information

The Sponsor is required to record the name and address of each IRB/IEC that reviews and approves this trial. The Sponsor is also required to document that each IRB/IEC meets regulatory and ICH GCP requirements by requesting and maintaining records of the names and qualifications of the IRB/IEC members and to make these records available for regulatory agency review upon request by those agencies.

10.2 Compliance with Financial Disclosure Requirements

Financial Disclosure requirements are outlined in the US Food and Drug Administration Regulations, Financial Disclosure by Clinical Investigators (21 CFR Part 54). It is the Sponsor's responsibility to determine, based on these regulations, whether a request for Financial Disclosure information is required. It is the investigator's/subinvestigator's responsibility to comply with any such request.

The investigator/subinvestigator(s) agree, if requested by the Sponsor in accordance with 21 CFR Part 54, to provide his/her financial interests in and/or arrangements with the Sponsor to allow for the submission of complete and accurate certification and disclosure statements.

The investigator/subinvestigator(s) further agree to provide this information on a Certification/Disclosure Form, commonly known as a financial disclosure form, provided by the Sponsor. The investigator/subinvestigator(s) also consent to the transmission of this information to the Sponsor in the United States for these purposes. This may involve the transmission of information to countries that do not have laws protecting personal data.

10.3 Compliance with Law, Audit and Debarment

By signing this protocol, the investigator agrees to conduct the trial in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical Practice (e.g., International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: Consolidated Guideline and other generally accepted standards of good clinical practice); and all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical trial.

The Code of Conduct, a collection of goals and considerations that govern the ethical and scientific conduct of clinical investigations sponsored by Merck, is provided in Section 12.1 - Merck Code of Conduct for Clinical Trials.

The investigator also agrees to allow monitoring, audits, IRB/ERC review and regulatory authority inspection of trial-related documents and procedures and provide for direct access to all trial-related source data and documents.

The investigator agrees not to seek reimbursement from subjects, their insurance providers or from government programs for procedures included as part of the trial reimbursed to the investigator by the Sponsor.

The investigator shall prepare and maintain complete and accurate trial documentation in compliance with Good Clinical Practice standards and applicable federal, state and local laws, rules and regulations; and, for each subject participating in the trial, provide all data, and, upon completion or termination of the clinical trial, submit any other reports to the Sponsor as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.

Trial documentation will be promptly and fully disclosed to the Sponsor by the investigator upon request and also shall be made available at the trial site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are requested by the Sponsor as a result of an audit to cure deficiencies in the trial documentation and worksheets/case report forms.

The investigator must maintain copies of all documentation and records relating to the conduct of the trial in compliance with all applicable legal and regulatory requirements. This documentation includes, but is not limited to, the protocol, worksheets/case report forms, advertising for subject participation, adverse event reports, subject source data, correspondence with regulatory authorities and IRBs/ERCs, consent forms, investigator's curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. By signing this protocol, the investigator agrees that documentation shall be retained until at least 2 years after the last

approval of a marketing application in an ICH region or until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Because the clinical development and marketing application process is variable, it is anticipated that the retention period can be up to 15 years or longer after protocol database lock. The Sponsor will determine the minimum retention period and notify the investigator when documents may be destroyed. The Sponsor will determine the minimum retention period and upon request, will provide guidance to the investigator when documents no longer need to be retained. The sponsor also recognizes that documents may need to be retained for a longer period if required by local regulatory requirements. All trial documents shall be made available if required by relevant regulatory authorities. The investigator must consult with and obtain written approval by the Sponsor prior to destroying trial and/or subject files.

ICH Good Clinical Practice guidelines recommend that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

The investigator will promptly inform the Sponsor of any regulatory authority inspection conducted for this trial.

Persons debarred from conducting or working on clinical trials by any court or regulatory authority will not be allowed to conduct or work on this Sponsor's trials. The investigator will immediately disclose in writing to the Sponsor if any person who is involved in conducting the trial is debarred or if any proceeding for debarment is pending or, to the best of the investigator's knowledge, threatened.

In the event the Sponsor prematurely terminates a particular trial site, the Sponsor will promptly notify that trial site's IRB/IEC.

According to European legislation, a Sponsor must designate an overall coordinating investigator for a multi-center trial (including multinational). When more than one trial site is open in an EU country, Merck, as the Sponsor, will designate, per country, a national principal coordinator (Protocol CI), responsible for coordinating the work of the principal investigators at the different trial sites in that Member State, according to national regulations. For a single-center trial, the Protocol CI is the principal investigator. In addition, the Sponsor must designate a principal or coordinating investigator to review the trial report that summarizes the trial results and confirm that, to the best of his/her knowledge, the report accurately describes the conduct and results of the trial [Clinical Study Report (CSR) CI]. The Sponsor may consider one or more factors in the selection of the individual to serve as the Protocol CI and or CSR CI (e.g., availability of the Protocol/CSR CI during the anticipated review process, thorough understanding of clinical trial methods, appropriate enrollment of subject cohort, timely achievement of trial milestones). The Protocol CI must be a participating trial investigator.

10.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Amendments Act (FDAAA) of 2007 and the European Medicines Agency (EMA) clinical trial Directive 2001/20/EC, the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to http://www.clinicaltrials.gov,

www.clinicaltrialsregister.eu or other local registries. Merck, as Sponsor of this trial, will review this protocol and submit the information necessary to fulfill these requirements. Merck entries are not limited to FDAAA or the EMA clinical trial directive mandated trials. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

By signing this protocol, the investigator acknowledges that the statutory obligations under FDAAA, the EMA clinical trials directive or other locally mandated registries are that of the Sponsor and agrees not to submit any information about this trial or its results to those registries.

10.5 Quality Management System

By signing this protocol, the Sponsor agrees to be responsible for implementing and maintaining a quality management system with written development procedures and functional area standard operating procedures (SOPs) to ensure that trials are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of Good Clinical Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical trial.

10.6 Data Management

The investigator or qualified designee is responsible for recording and verifying the accuracy of subject data. By signing this protocol, the investigator acknowledges that his/her electronic signature is the legally binding equivalent of a written signature. By entering his/her electronic signature, the investigator confirms that all recorded data have been verified as accurate.

Detailed information regarding Data Management procedures for this protocol will be provided separately.

10.7 Publications

This trial is intended for publication, even if terminated prematurely. Publication may include any or all of the following: posting of a synopsis online, abstract and/or presentation at a scientific conference, or publication of a full manuscript. The Sponsor will work with the authors to submit a manuscript describing trial results within 12 months after the last data become available, which may take up to several months after the last subject visit in some cases such as vaccine trials. However, manuscript submission timelines may be extended on OTC trials. For trials intended for pediatric-related regulatory filings, the investigator agrees to delay publication of the trial results until the Sponsor notifies the investigator that all relevant regulatory authority decisions on the trial drug have been made with regard to pediatric-related regulatory filings. Merck will post a synopsis of trial results for approved products on www.clinicaltrials.gov by 12 months after the last subject's last visit for the primary outcome, 12 months after the decision to discontinue development, or product marketing (dispensed, administered, delivered or promoted), whichever is later.

These timelines may be extended for products that are not yet marketed, if additional time is needed for analysis, to protect intellectual property, or to comply with confidentiality

agreements with other parties. Authors of the primary results manuscript will be provided the complete results from the Clinical Study Report, subject to the confidentiality agreement. When a manuscript is submitted to a biomedical journal, the Sponsor's policy is to also include the protocol and statistical analysis plan to facilitate the peer and editorial review of the manuscript. If the manuscript is subsequently accepted for publication, the Sponsor will allow the journal, if it so desires, to post on its website the key sections of the protocol that are relevant to evaluating the trial, specifically those sections describing the trial objectives and hypotheses, the subject inclusion and exclusion criteria, the trial design and procedures, the efficacy and safety measures, the statistical analysis plan, and any amendments relating to those sections. The Sponsor reserves the right to redact proprietary information.

For multicenter trials, subsequent to the multicenter publication (or after public disclosure of the results online at www.clinicaltrials.gov if a multicenter manuscript is not planned), an investigator and his/her colleagues may publish their data independently. In most cases, publication of individual trial site data does not add value to complete multicenter results, due to statistical concerns. In rare cases, publication of single trial site data prior to the main paper may be of value. Limitations of single trial site observations in a multicenter trial should always be described in such a manuscript.

Authorship credit should be based on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors must meet conditions 1, 2 and 3. Significant contributions to trial execution may also be taken into account to determine authorship, provided that contributions have also been made to all three of the preceding authorship criteria. Although publication planning may begin before conducting the trial, final decisions on authorship and the order of authors' names will be made based on participation and actual contributions to the trial and writing, as discussed above. The first author is responsible for defending the integrity of the data, method(s) of data analysis and the scientific content of the manuscript.

The Sponsor must have the opportunity to review all proposed abstracts, manuscripts or presentations regarding this trial 45 days prior to submission for publication/presentation. Any information identified by the Sponsor as confidential must be deleted prior to submission; this confidentiality does not include efficacy and safety results. Sponsor review can be expedited to meet publication timelines.

11.0 LIST OF REFERENCES

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12.0 APPENDICES

12.1 Merck Code of Conduct for Clinical Trials

Merck*
Code of Conduct for Clinical Trials

I. Introduction

A. Purpose

Merck, through its subsidiaries, conducts clinical trials worldwide to evaluate the safety and effectiveness of our products. As such, we are committed to designing, implementing, conducting, analyzing and reporting these trials in compliance with the highest ethical and scientific standards. Protection of subject safety is the overriding concern in the design of clinical trials. In all cases, Merck clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

B. Scope

Such standards shall be endorsed for all clinical interventional investigations sponsored by Merck irrespective of the party (parties) employed for their execution (e.g., contract research organizations, collaborative research efforts). This Code is not intended to apply to trials which are observational in nature, or which are retrospective. Further, this Code does not apply to investigator-initiated trials which are not under the control of Merck.

II. Scientific Issues

A. Trial Conduct

1. Trial Design

Except for pilot or estimation trials, clinical trial protocols will be hypothesis-driven to assess safety, efficacy and/or pharmacokinetic or pharmacodynamic indices of Merck or comparator products. Alternatively, Merck may conduct outcomes research trials, trials to assess or validate various endpoint measures, or trials to determine subject preferences, etc.

The design (i.e., subject population, duration, statistical power) must be adequate to address the specific purpose of the trial. Research subjects must meet protocol entry criteria to be enrolled in the trial.

2. Site Selection

Merck selects investigative sites based on medical expertise, access to appropriate subjects, adequacy of facilities and staff, previous performance in Merck trials, as well as budgetary considerations. Prior to trial initiation, sites are evaluated by Merck personnel to assess the ability to successfully conduct the trial.

3. Site Monitoring/Scientific Integrity

Trial sites are monitored to assess compliance with the trial protocol and general principles of Good Clinical Practice. Merck reviews clinical data for accuracy, completeness and consistency. Data are verified versus source documentation according to standard operating procedures. Per Merck policies and procedures, if fraud, misconduct or serious GCP-non-Compliance are suspected, the issues are promptly investigated. When necessary, the clinical site will be closed, the responsible regulatory authorities and ethics review committees notified and data disclosed accordingly.

B. Publication and Authorship

To the extent scientifically appropriate, Merck seeks to publish the results of trials it conducts. Some early phase or pilot trials are intended to be hypothesis-generating rather than hypothesis testing. In such cases, publication of results may not be appropriate since the trial may be underpowered and the analyses complicated by statistical issues of multiplicity.

Merck's policy on authorship is consistent with the requirements outlined in the ICH-Good Clinical Practice guidelines. In summary, authorship should reflect significant contribution to the design and conduct of the trial, performance or interpretation of the analysis, and/or writing of the manuscript. All named authors must be able to defend the trial results and conclusions. Merck funding of a trial will be acknowledged in publications.

III. Subject Protection

A. IRB/ERC review

All clinical trials will be reviewed and approved by an independent IRB/ERC before being initiated at each site. Significant changes or revisions to the protocol will be approved by the IRB/ERC prior to implementation, except that changes required urgently to protect subject safety and well-being may be enacted in anticipation of IRB/ERC approval. For each site, the IRB/ERC and Merck will approve the subject informed consent form.

B. Safety

The guiding principle in decision-making in clinical trials is that subject welfare is of primary importance. Potential subjects will be informed of the risks and benefits of, as well as alternatives to, trial participation. At a minimum, trial designs will take into account the local standard of care. Subjects are never denied access to appropriate medical care based on participation in a Merck clinical trial.

All participation in Merck clinical trials is voluntary. Subjects are enrolled only after providing informed consent for participation. Subjects may withdraw from a Merck trial at any time, without any influence on their access to, or receipt of, medical care that may otherwise be available to them.

C. Confidentiality

Merck is committed to safeguarding subject confidentiality, to the greatest extent possible. Unless required by law, only the investigator, sponsor (or representative) and/or regulatory authorities will have access to confidential medical records that might identify the research subject by name.

D. Genomic Research

Genomic Research will only be conducted in accordance with informed consent and/or as specifically authorized by an Ethics Committee.

IV. Financial Considerations

A. Payments to Investigators

Clinical trials are time- and labor-intensive. It is Merck's policy to compensate investigators (or the sponsoring institution) in a fair manner for the work performed in support of Merck trials. Merck does not pay incentives to enroll subjects in its trials. However, when enrollment is particularly challenging, additional payments may be made to compensate for the time spent in extra recruiting efforts.

Merck does not pay for subject referrals. However, Merck may compensate referring physicians for time spent on chart review to identify potentially eligible subjects.

B. Clinical Research Funding

Informed consent forms will disclose that the trial is sponsored by Merck, and that the investigator or sponsoring institution is being paid or provided a grant for performing the trial. However, the local IRB/ERC may wish to alter the wording of the disclosure statement to be consistent with financial practices at that institution. As noted above, publications resulting from Merck trials will indicate Merck as a source of funding.

C. Funding for Travel and Other Requests

Funding of travel by investigators and support staff (e.g., to scientific meetings, investigator meetings, etc.) will be consistent with local guidelines and practices including, in the U.S., those established by the American Medical Association (AMA).

V. Investigator Commitment

Investigators will be expected to review Merck's Code of Conduct as an appendix to the trial protocol, and in signing the protocol, agree to support these ethical and scientific standards.

* In this document, "Merck" refers to Merck Sharp & Dohme Corp. and Schering Corporation, each of which is a subsidiary of Merck & Co., Inc. Merck is known as MSD outside of the United States and Canada. As warranted by context, Merck also includes affiliates and subsidiaries of Merck & Co., Inc."

12.2 Collection and Management of Specimens for Future Biomedical Research

1. Definitions

a. Biomarker: A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition.[1]

b. Pharmacogenomics: The investigation of variations of DNA and RNA characteristics as related to drug/vaccine response.[2]

c. Pharmacogenetics: A subset of pharmacogenomics, pharmacogenetics is the influence of variations in DNA sequence on drug/vaccine response.[2]

d. DNA: Deoxyribonucleic acid.

e. RNA: Ribonucleic acid.

2. Scope of Future Biomedical Research

The specimens consented and/or collected in this trial as outlined in Section 7.1.3.7 – Future Biomedical Research Samples will be used in various experiments to understand:

- The biology of how drugs/vaccines work
- Biomarkers responsible for how a drug/vaccine enters and is removed by the body
- Other pathways drugs/vaccines may interact with
- The biology of disease

The specimen(s) may be used for future assay development and/or drug/vaccine development.

It is now well recognized that information obtained from studying and testing clinical specimens offers unique opportunities to enhance our understanding of how individuals respond to drugs/vaccines, enhance our understanding of human disease and ultimately improve public health through development of novel treatments targeted to populations with the greatest need. All specimens will be used by the Sponsor or those working for or with the Sponsor.

3. Summary of Procedures for Future Biomedical Research

a. Subjects for Enrollment

All subjects enrolled in the clinical trial will be considered for enrollment in the Future Biomedical Research sub-trial.

b. Informed Consent

Informed consent for specimens (i.e., DNA, RNA, protein, etc.) will be obtained during screening for protocol enrollment from all subjects or legal guardians, at a trial visit by the investigator or his or her designate. Informed consent for Future Biomedical Research should be presented to the subjects on the visit designated in the trial flow chart. If delayed, present consent at next possible Subject Visit. Consent forms signed by the subject will be kept at the clinical trial site under secure storage for regulatory reasons.

A template of each trial site's approved informed consent will be stored in the Sponsor's clinical document repository.

c. eCRF Documentation for Future Biomedical Research Specimens

Documentation of subject consent for Future Biomedical Research will be captured in the electronic Case Report Forms (eCRFs). Any specimens for which such an informed consent cannot be verified will be destroyed.

d. Future Biomedical Research Specimen(s)

Collection of specimens for Future Biomedical Research will be performed as outlined in the trial flow chart. In general, if additional blood specimens are being collected for Future Biomedical Research, these will usually be obtained at a time when the subject is having blood drawn for other trial purposes.

4. Confidential Subject Information for Future Biomedical Research

In order to optimize the research that can be conducted with Future Biomedical Research specimens, it is critical to link subject' clinical information with future test results. In fact little or no research can be conducted without connecting the clinical trial data to the specimen. The clinical data allow specific analyses to be conducted. Knowing subject characteristics like gender, age, medical history and treatment outcomes are critical to understanding clinical context of analytical results.

To maintain privacy of information collected from specimens obtained for Future Biomedical Research, the Sponsor has developed secure policies and procedures. All specimens will be single-coded per ICH E15 guidelines as described below.

At the clinical trial site, unique codes will be placed on the Future Biomedical Research specimens. This code is a random number which does not contain any personally identifying information embedded within it. The link (or key) between subject identifiers and this unique code will be held at the trial site. No personal identifiers will appear on the specimen tube.

5. Biorepository Specimen Usage

Specimens obtained for the Sponsor will be used for analyses using good scientific practices. Analyses utilizing the Future Biomedical Research specimens may be performed by the Sponsor, or an additional third party (e.g., a university investigator) designated by the Sponsor. The investigator conducting the analysis will follow the Sponsor's privacy and confidentiality requirements. Any contracted third party analyses will conform to the specific scope of analysis outlined in this sub-trial. Future Biomedical Research specimens remaining with the third party after specific analysis is performed will be reported to the Sponsor.

6. Withdrawal From Future Biomedical Research

Subjects may withdraw their consent for Future Biomedical Research and ask that their biospecimens not be used for Future Biomedical Research. Subjects may withdraw consent at any time by contacting the principal investigator for the main trial. If medical records for the main trial are still available, the investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com).

Subsequently, the subject's specimens will be flagged in the biorepository and restricted to main study use only. If specimens were collected from study participants specifically for Future Biomedical Research, these specimens will be removed from the biorepository and destroyed. Documentation will be sent to the investigator confirming withdrawal and/or destruction, if applicable. It is the responsibility of the investigator to inform the subject of completion of the withdrawal and/or destruction, if applicable. Any analyses in progress at the time of request for withdrawal/destruction or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research trial data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main trial are no longer available (e.g., if the investigator is no longer required by regulatory authorities to retain the main trial records) or the specimens have been completely anonymized, there will no longer be a link between the subject's personal information and their specimens. In this situation, the request for withdrawal of consent and/or destruction cannot be processed.

7. Retention of Specimens

Future Biomedical Research specimens will be stored in the biorepository for potential analysis for up to 20 years from the end of the main study. Specimens may be stored for longer if a regulatory or governmental authority has active questions that are being answered. In this special circumstance, specimens will be stored until these questions have been adequately addressed.

Specimens from the trial site will be shipped to a central laboratory and then shipped to the Sponsor-designated biorepository. If a central laboratory is not utilized in a particular trial, the trial site will ship directly to the Sponsor-designated biorepository. The specimens will be stored under strict supervision in a limited access facility which operates to assure the integrity of the specimens. Specimens will be destroyed according to Sponsor policies and procedures and this destruction will be documented in the biorepository database.

8. Data Security

Databases containing specimen information and test results are accessible only to the authorized Sponsor representatives and the designated trial administrator research personnel and/or collaborators. Database user authentication is highly secure, and is accomplished using network security policies and practices based on international standards to protect against unauthorized access.

9. Reporting of Future Biomedical Research Data to Subjects

No information obtained from exploratory laboratory studies will be reported to the subject, family, or physicians. Principle reasons not to inform or return results to the subject include: Lack of relevance to subject health, limitations of predictive capability, and concerns regarding misinterpretation.

If important research findings are discovered, the Sponsor may publish results, present results in national meetings, and make results accessible on a public website in order to rapidly report this information to doctors and subjects. Subjects will not be identified by

name in any published reports about this study or in any other scientific publication or presentation.

10. Future Biomedical Research Study Population

Every effort will be made to recruit all subjects diagnosed and treated on Sponsor clinical trials for Future Biomedical Research.

11. Risks Versus Benefits of Future Biomedical Research

For future biomedical research, risks to the subject have been minimized. Risks include those associated with venipuncture to obtain the whole blood specimen. This specimen will be obtained at the time of routine blood specimens drawn in the main trial.

The Sponsor has developed strict security, policies and procedures to address subject data privacy concerns. Data privacy risks are largely limited to rare situations involving possible breach of confidentiality. In this highly unlikely situation there is risk that the information, like all medical information, may be misused.

12. Questions

Any questions related to the future biomedical research should be e-mailed directly to clinical.specimen.management@merck.com.

13. References

1. National Cancer Institute: http://www.cancer.gov/dictionary/?searchTxt=biomarker
2. International Conference on Harmonization: DEFINITIONS FOR GENOMIC BIOMARKERS, PHARMACOGENOMICS, PHARMACOGENETICS, GENOMIC DATA AND SAMPLE CODING CATEGORIES - E15; http://www.ich.org/LOB/media/MEDIA3383.pdf
3. Industry Pharmacogenomics Working Group. Understanding the Intent, Scope and Public Health Benefits of Exploratory Biomarker Research: A Guide for IRBs/IECs and Investigational Site Staff. Available at http://i-pwg.org/
4. Industry Pharmacogenomics Working Group. Pharmacogenomics Informational Brochure for IRBs/IECs and Investigational Site Staff. Available at http://i-pwg.org/

12.3 Approximate Blood Volumes Drawn/Collected by Trial Visit and Sample Types

Trial Visit	Screening	Visit 1 (Dose 1)	Visit 2 7 days PD1	Visit 3 12 days PD1	Visit 4 28 days PD1	Visit 5 56 days PD1	Visit 6 Month 6 PD1/(Dose 2)	Visit7 7 days PD2	Visit 8 12 days PD2	Visit 9 28 days PD2	Visit 10 Month 6 PD2	Visit 11 1 Year PD2	Total
Blood Parameter		**Approximate Blood Volume (mL)**											
Blood Sample for Screening[1]	36												36
Blood sample for laboratory testing (with viremia tested at 7, 12, and 28 days following each vaccination)		16	21	21	21		16	21	21	21			158
Blood (DNA) for Future Biomedical Research		8.5											8.5
Serum[2] samples for flavivirus testing (Dengue IgG ELISA)		4											4
Serum[2] samples for antibody responses (VRNT)		5			5	5	5			5	5	5	35
Serum[2] samples for assay development		15			15		15						45
Expected Total (mL)	36	48.5	21	21	21	5	36	21	21	21	5	5	286.5

[1] Sample may for the following tests: Hematology / chemistry; HIV, Hepatitis B, and Hepatitis C; Pregnancy testing; Alcohol testing (blood or breath testing); and Hemoglobin A1c (HbA1c)

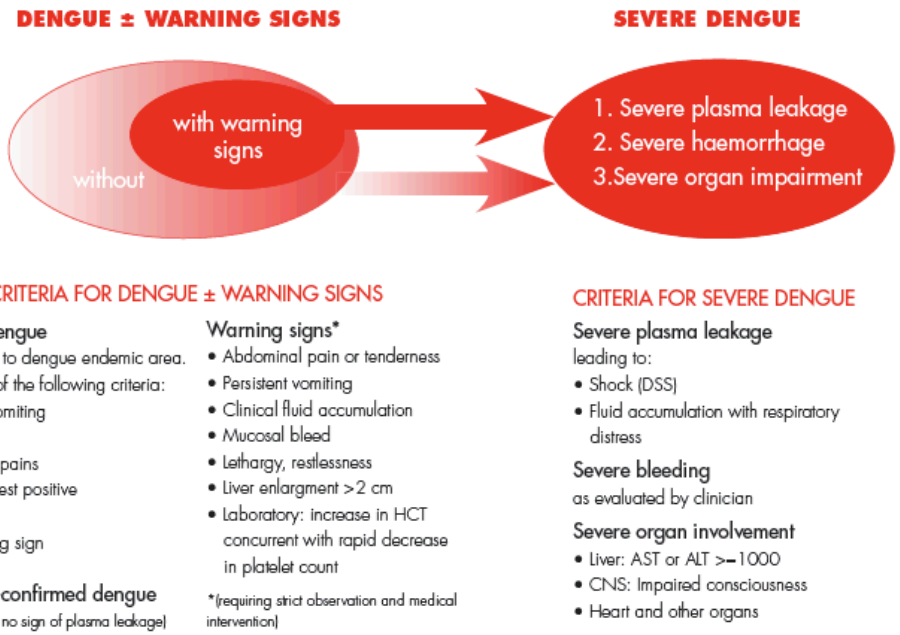
[2] Note: Volumes are for blood samples; the testing will be completed on serum but blood volumes are provided for purposes of blood draw estimates.
PD1 = Postdose 1; PD2 = Postdose 2; VRNT = Virus Reduction Neutralization Test; DNA = deoxyribonucleic acid; IgG-ELISA = immunoglobulin G-Enzyme-Linked Immunosorbent Assay; mL = milliliter

Subjects Requiring Evaluation for Suspected Dengue (Unscheduled Visits)

Trial Visit	Acute Initial Evaluation Visit	Convalescent Follow-up Visit 14 to 28 Days After Acute Visit	Dengue Evaluation Visits Total
Blood Parameter	**Approximate Blood Volume (mL)**		
Specimen collection for safety labs (chemistry, hematology, urinalysis)	16	16	32
Serum sample for zika and chikungunya testing (PCR)	8		8
Serum sample for virological confirmation of dengue with qualitative and/or quantitative serotype-specific RT-PCR	5		5
Serum sample for dengue antibody test with VRNT		5	5
Expected Total (mL)	29	21	50

VRNT = Virus Reduction Neutralization Test; PCR = polymerase chain reaction; RT-PCR = reverse transcriptase-polymerase chain reaction; mL = milliliter

12.4 2009 WHO Dengue Guidelines for Diagnosis, Treatment, Prevention and Control



Source: [1]

12.5 LIST OF ABBREVIATIONS

AEs	Adverse events
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ASaT	All subjects as treated
β-hCG	Beta-human chorionic gonadotropin
BMI	Body mass index
CSR	Clinical Study Report
DALYs	Disability-adjusted life-years
DEG	Data entry guideline
DENV	Dengue virus
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
eCRF	Electronic case report form
ERC	Ethics Review Committee
eVRC	Electronic Vaccination Report Card
FAS	Full Analysis Set
FBR	Future biomedical research
GCP	Good Clinical Practice
GMFRs	Geometric mean fold ratios
GMT	Geometric mean titer
HBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus
HQ	Headquarters
IA	Interim analysis
IB	Investigator's Brochure
ICF	Informed consent form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive response technology
IVRS	Interactive voice response system
LATV	Live-attenuated tetravalent vaccine
LID	Laboratories of Infectious Disease
LLN	Lower limit of normal
NIH	National Institutes of Health
NIAID	National Institute of Allergy and Infectious Diseases
NT	Neutralization Test
OD	Optical density
PCR	Polymerase chain reaction
PD1	Postdose 1
PD2	Postdose 2
PFU	Plaque forming units
RT-PCR	Reverse transcription polymerase chain reaction
SAE	Serious adverse event/experience
siDMC	Standing Internal Data Monitoring Committee
SOC	System Organ Class
SOPs	Standard operating procedures

sSAP	Supplemental Statistical Analysis Plan
ULN	Upper limit of normal
US	United States
VCD	Virologically-confirmed dengue
VRNT	Virus Reduction Neutralization Test
WHO	World Health Organization

12.6 Adverse Events Toxicity Grading Scale

A toxicity grading scale will be assigned to all clinical and laboratory adverse events as displayed in the tables below.

Injection-site Adverse Event Toxicity Grading Scale

Injection-site Reaction to Study Vaccine/Placebo*	Grade 1	Grade 2	Grade 3	Grade 4
Injection-site AEs occurring Days 1 through 5 following receipt of study vaccine/placebo				
Pain/Tenderness	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Erythema/Redness	Size measured as B	Size measured as C or D	Size measured as E	Necrosis or exfoliative dermatitis or results in ER visit or hospitalization
Induration/Swelling	Size measured as B	Size measured as C or D	Size measured as E	Necrosis or ER visit or hospitalization
Other	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Any injection-site reaction that begins ≥ 6 days after receipt of study vaccine/placebo				
Pain/tenderness Erythema/Redness Induration/Swelling Other	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
*Based upon information provided by the patient on the electronic Vaccine Report Card (eVRC) and verbally during eVRC review. Erythema/Redness/Induration and Swelling are specific injection-site AEs with size designations of letters A through E, based upon a graphic in the eVRC. Size A is not assigned a toxicity grade; however, injection-site AEs that measure size A should be reported as adverse events. If the patient has an ER visit or is hospitalized for any injection-site AE, that AE is to be assigned a toxicity grade of 4, regardless of the size measured.				

Systemic Adverse Event Toxicity Grading Scale

Vital Signs *	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) ** (°F) **	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***	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

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** 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.

Systemic (General)	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Systemic Adverse Event Toxicity Grading Scale (continued)

Systemic Illnes	Mild (Grade 1)	(Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

Laboratory Adverse Event Toxicity Grading Scale

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

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia 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***	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

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

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

***ULN' is the upper limit of the normal range.

Laboratory Adverse Event Toxicity Grading Scale (continued)

Hematology *	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**	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)

* 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.
** "ULN" is the upper limit of the normal range.

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

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

The toxicity grading scales for systemic and laboratory adverse events are from "Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" FDA 2007. [32]

13.0 SIGNATURES

13.1 Sponsor's Representative

TYPED NAME	
TITLE	
SIGNATURE	
DATE SIGNED	

13.2 Investigator

I agree to conduct this clinical trial in accordance with the design outlined in this protocol and to abide by all provisions of this protocol (including other manuals and documents referenced from this protocol). I agree to conduct the trial in accordance with generally accepted standards of Good Clinical Practice. I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse events as defined in Section 7.0 – TRIAL PROCEDURES (Assessing and Recording Adverse Events). I also agree to handle all clinical supplies provided by the Sponsor and collect and handle all clinical specimens in accordance with the protocol. I understand that information that identifies me will be used and disclosed as described in the protocol, and that such information may be transferred to countries that do not have laws protecting such information. Since the information in this protocol and the referenced Investigator's Brochure is confidential, I understand that its disclosure to any third parties, other than those involved in approval, supervision, or conduct of the trial is prohibited. I will ensure that the necessary precautions are taken to protect such information from loss, inadvertent disclosure or access by third parties.

TYPED NAME	
TITLE	
SIGNATURE	
DATE SIGNED	