Clinical Study Protocol

NCT Number: NCT03746015

Title: An Open-Label, Phase 2 Trial to Investigate the Humoral and Cell-Mediated Immune Responses and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Flavivirus-Naïve and Dengue-Immune Healthy Adults

Study Number: DEN-210

Document Version and Date: Version 5.0, 18 December 2020

Certain information within this document has been redacted (ie, specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

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16.1.1 Protocol and Protocol Amendments

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1.3 Protocol Amendment Summary of Changes

This document describes the changes in reference to the Protocol incorporating Amendment No. 1.

1.3.1 Amendment History

Date	Amendment Number	Amendment Type	Region
02 May 2018	Initial Protocol	Not applicable	Local (United States of America)
23 January 2019	1	Non-substantial	Local (United States of America)

1.3.2 Summary of Changes

Amendment to Protocol Version 1.0 dated 02 May 2018	
Rationale for the Amendment: This protocol has been amended to implement modifications with the aim of accelerating the recruitment process and to anticipate on subject drop-outs. In particular, participation of more than one trial center has been made possible and the overall sample size is increased by 10% to 22 subjects in each trial group, with the aim of 20 subjects being evaluable in each trial group. Other modifications Trial days 93, 95, 98, 101, and 104 have been corrected into trial days 94, 96, 99, 102 and 105, respectively. Update of literature list. Administrative changes. Minor grammatical and editorial changes are included for clarification and consistency purposes only. New text is shown in bold italics and any deleted text is marked using strikethrough.	
Section	**Description of change**
Title page	Date: ~~02 May 2018~~ ***23 January 2019*** Version: Version ~~1.0~~ ***2.0 (supersedes Version 1.0)***
Section 1.2	**SIGNATURES** [redacted], PhD, MSc PV [redacted] Takeda Pharmaceuticals International AG [redacted] ~~, HN BC~~ [redacted] ***, MPH*** [redacted] Vaccine~~s~~ Business Unit Takeda Vaccines, Inc. [redacted], MD [redacted], ~~Dengue~~ Clinical Development Vaccine Business Unit Takeda Pharmaceuticals ~~International AG~~ ***USA, Inc.***

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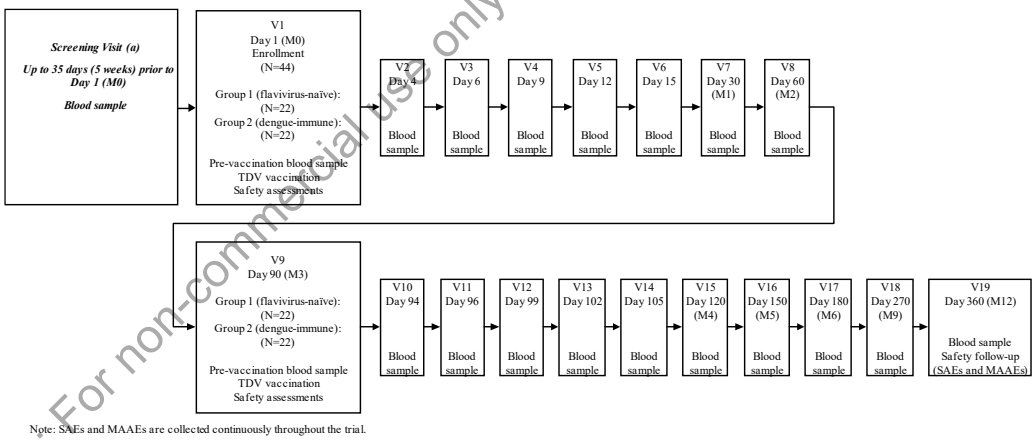
Section	Description of change
Sections 2.0 and 4.2	This trial will be conducted at ~~a single~~ trial center***s*** in the United States of America ~~(USA)~~ ~~using a pre-existing pool of~~ ***involving*** healthy adults who are either flavivirus-naïve or seropositive for DENV-1 or DENV-3 (ie, serology consistent with primary infection with either DENV-1 or DENV-3). The serological status of subjects ***will either*** ~~has~~ ***have*** been determined by the trial center prior to and outside the scope of this trial ***or will be determined through screening within the scope of this trial***.
Sections 2.0, 6.1	This is an open-label, phase 2 trial in ~~40~~ ***44*** healthy adult subjects aged 18 to 60 years (inclusive) to investigate the immunogenicity and safety of SC administration of a 2-dose regimen of TDV. Subjects will be enrolled in 2 trial groups based on results from serological testing performed ***either*** by the trial center~~s~~ ***prior to and*** outside the scope of this trial ***or through screening within the scope of this trial*** (up to 35 days [5 weeks] prior to Day 1[M0]): – Group 1: approximately ~~20~~ ***22*** flavivirus-naïve subjects. – Group 2: approximately ~~20~~ ***22*** dengue-immune subjects (ie, approximately ~~10~~ ***11*** subjects with serology consistent with primary infection by DENV-1 and approximately ~~10~~ ***11*** subjects with serology consistent with primary infection by DENV-3).  Note: SAEs and MAAEs are collected continuously throughout the trial. M=month, MAAE=medically attended adverse event, SAE=serious adverse event, V=visit ***(a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.***
	All subjects will be followed-up for 9 months post second vaccination so the trial duration will be approximately 360 days (12 months) ***or 13 months*** for each subject ***depending on whether serological testing with regard to the inclusion criteria for the trial is performed outside or within the scope of this trial, respectively.***
Section 2.0	**Subject Population:** Planned Number of Subjects: ~~40~~ ***44*** Planned Number of Trial Arms: 2 trial groups (~~20~~ ***22*** subjects per trial group); 2-dose regimen (1 trial vaccine dose on Day 1 [M0] and 1 trial vaccine dose on Day 90 [M3]), SC route. Estimated Total: ~~40~~ ***44*** enrolled subjects.

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<table>
<tr><th>Section</th><th>Description of change</th></tr>
<tr><td>Sections 2.0 and 7.1</td><td>4. Group 1 only: immunologically naïve to dengue, Zika, Yellow Fever (YF), Japanese Encephalitis (JE), West Nile (WN) (based on negative results for detection of anti-DENV, anti-Zika, anti-YF, anti-JE, anti-WN antibodies) as documented by serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 35 days [5 weeks] prior to Day 1 [M0]).
5. Group 2 only: serology consistent with primary infection with either DENV-1 or DENV-3 (defined as detectable neutralizing antibodies against DENV-1 or DENV-3 only, or titers for DENV-1 or DENV-3 ≥4-times higher than titers for the 2 other dengue serotypes) as documented by serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 35 days [5 weeks] prior to Day 1 [M0]).</td></tr>
<tr><td>Section 2.1</td><td>Table 2.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 [M12])
<table>
<tr><td colspan="2"></td><td>Screening visit (a)</td><td>V1</td></tr>
<tr><td colspan="2"></td><td>Up to 35 days (5 weeks) prior to D1 (M0)</td><td>D1
M0</td></tr>
<tr><td colspan="2">Informed consent[~~a~~]</td><td>X</td><td>X (b)</td></tr>
<tr><td colspan="2">Assessment of eligibility criteria[~~bc~~]</td><td>X</td><td>X</td></tr>
<tr><td colspan="2">Trial group assignment (flavivirus-naïve or dengue-immune)[~~cd~~]</td><td></td><td>X</td></tr>
<tr><td colspan="2">Demographics~~, medical history, and prior medication/ vaccination~~</td><td>X</td><td>X(e)</td></tr>
<tr><td colspan="2">Medical history</td><td>X</td><td>X</td></tr>
<tr><td colspan="2">Prior medication/vaccination</td><td>X</td><td>X</td></tr>
<tr><td colspan="2">Concomitant medications/vaccinations[~~df~~]</td><td>X</td><td>X</td></tr>
<tr><td colspan="2">Review of systems</td><td></td><td>X</td></tr>
<tr><td colspan="2">Complete physical examination[~~eg~~]</td><td>X</td><td>X</td></tr>
<tr><td colspan="2">Targeted physical examination[~~fh~~]</td><td></td><td>X</td></tr>
<tr><td colspan="2">Vital signs[~~gi~~]</td><td></td><td></td></tr>
<tr><td colspan="2">Pregnancy test[~~hj~~]</td><td>X</td><td>X</td></tr>
<tr><td colspan="2">Pregnancy avoidance guidance[~~ik~~]</td><td></td><td>X</td></tr>
<tr><td colspan="2">Blood sampling for serological testing (2.5 mL)</td><td>X</td><td>X</td></tr>
<tr><td colspan="2">Blood sample for humoral immune response (20 mL)[~~jl, km~~]</td><td></td><td></td></tr>
<tr><td colspan="2">Blood sample for T cell-mediated immune response (60 mL)[~~jl~~]</td><td></td><td>X</td></tr>
<tr><td colspan="2">Blood sample for B cell-mediated immune response (30 mL)[~~jl~~]</td><td></td><td>X</td></tr>
<tr><td colspan="2">Blood sample for innate immune response (5 mL)[~~jl~~]</td><td></td><td>X</td></tr>
<tr><td colspan="2">Blood sample for vaccine viremia (5 mL)[~~jl~~]</td><td></td><td>X</td></tr>
<tr><td colspan="2">Check criteria for delay of trial vaccine administration</td><td></td><td>X</td></tr>
<tr><td colspan="2">Check contraindications for trial vaccine administration</td><td></td><td>X</td></tr>
<tr><td colspan="2">Trial vaccine administration[~~ln~~]</td><td></td><td>X</td></tr>
<tr><td colspan="2">Injection site evaluation[~~mo~~]</td><td></td><td>X</td></tr>
<tr><td rowspan="2">Diary card [~~np~~]</td><td>Distribution</td><td></td><td>X</td></tr>
<tr><td>Review/collection of solicited and unsolicited AEs</td><td></td><td>X</td></tr>
<tr><td colspan="2">AEs leading to trial vaccine withdrawal or trial discontinuation, SAEs, MAAEs[~~eq~~]</td><td></td><td>X</td></tr>
<tr><td colspan="4">AEs=Adverse Events, D=Day, M=Month, MAAEs=Medically Attended Adverse Events, NA=Not Applicable, SAEs= Serious Adverse Events, V=Visit
(a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.
(b) Up to 28 days prior to Day 1 (Month 0 [M0]).</td></tr>
</table></td></tr>
</table>

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Section	Description of change
Section 2.1	***(c)*** ~~(b)~~ After informed consent has been obtained, eligibility of the subject will be assessed by review of inclusion/exclusion criteria ***at Screening or*** prior to TDV administration on Day 1 (M0)***, as applicable***. ***(d)*** ~~(c)~~ Subjects will be enrolled in Group 1 (flavivirus-naïve subjects) or Group 2 (dengue-immune subjects with serology consistent with primary infection with either wild type dengue virus [DENV]-1 or DENV-3) based on serological testing performed ***either*** by the trial center ***prior to and*** outside the scope of this trial ***or within the scope of this trial*** (up to 35 days [5 weeks] prior to Day 1 [M0]). ***(e)*** ***Not applicable if a Screening visit has been performed.*** ***(f)*** ~~(d)~~ All medications and vaccine history from 1 month (minimum 28 days) prior to administration of each trial vaccine dose up to 1 month (minimum 28 days) thereafter, steroids and immunostimulants within 60 days prior to Day 1 (M0), immunoglobulins and blood products within 3 months prior to Day 1 (M0), and immunosuppressive therapy within 6 months prior to Day 1 (M0). Concomitant medication/vaccination will be collected throughout the trial. ***(g)*** ~~(e)~~ Physical examination including measurement of weight and height; Body Mass Index (BMI) will be calculated. Measurement of height is only required at ***Screening or at*** Day 1 (M0), ***as applicable***. ***(h)*** ~~(f)~~ Subjects may undergo a targeted symptom-directed physical examination. Clinically significant changes from the Baseline examination should be recorded in the subject's source documents and electronic Case Report Form (eCRF). ***(i)*** ~~(g)~~ Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature. ***(j)*** ~~(h)~~ Pregnancy testing (serum or urine) for females of childbearing potential. Results must be confirmed and documented as negative prior to each trial dose administration. Additional pregnancy tests may be performed during the trial if deemed necessary by the investigator. ***(k)*** ~~(i)~~ Females of childbearing potential who are sexually active will be reminded during trial visits to adhere to acceptable contraceptive methods up to 6 weeks after the last dose of TDV (Day 90 [Month 3] (M3]) + 6 weeks). Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process and will be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy and donation of ova. During the course of the trial, subjects of childbearing potential will receive continued guidance with respect to the avoidance of pregnancy. ***(l)*** ~~(j)~~ All blood samples on days of vaccination (Day 1 [M0] and Day 90 [M3]) should be taken prior to administration of TDV. ***(m)*** ~~(k)~~ Dengue neutralizing antibodies and anti-dengue Non-Structural protein 1 (NS1) antibodies for all subjects, and T cell epitope mapping in a subset of subjects with Interferon-gamma Enzyme-Linked Immunospot (IFN- γELISpot) responses >500 Spot Forming Cells/106 cells and availability of sufficient cells). ***(n)*** ~~(l)~~ Subjects will receive TDV by subcutaneous injection. ***(o)*** ~~(m)~~ Injection site pain, erythema, and swelling assessed by trial staff for 30 minutes post-vaccination. ***(p)*** ~~(n)~~ Diary cards (paper or electronic) will be distributed for the recording of 1) solicited AEs including solicited local (injection site) reactions for 7 days (day of administration + 6 days) and solicited systemic events for 14 days (day of administration + 13 days) following administration of each trial vaccine dose, and 2) unsolicited AE for 28 days (day of administration + 27 days) following administration of each trial vaccine dose. The investigator will categorize events by severity (mild, moderate or severe) and will assess causality to vaccine administration ("related" or "not related"). For persistent/prolonged solicited local (injection site) reactions or systemic events observed as continuing on Day 8 or 15, respectively, following each trial vaccination, the end date will be captured on the "Adverse Event" eCRF to permit a separate analysis from the unsolicited AEs. Any solicited local (injection site) reaction or systemic event that resolves before 8 or 15 days, respectively, following each trial vaccination, but recurs at a later time (ie, if discontinues), should be recorded as an unsolicited AE on the "Adverse Event" eCRF. ***(q)*** ~~(o)~~ MAAEs and SAEs will be collected for the trial duration. ***(r)*** ~~(p)~~ The Final Visit will be performed on Day 360 (Month 12 [M12]). If a subject terminates trial participation earlier, Day 360 (M12) procedures should be performed at their last trial visit, if possible.
	V19 D360 (~~p~~ ***r***) M12
Sections 2.0 and 6.3	The trial duration for each subject will be ***at least*** approximately 360 days (12 months) including vaccination (Day 1 [M0] and Day 90 [M3]) and follow-up through Day 360 (M12). ***This duration will be approximately 13 months if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial (ie, up to 35 days [5 weeks] prior to Day 1 [M0]).***

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Section	Description of change
Sections 1.2, 4.2, 15.0, and 15.2	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6 Good Clinical Practice (GCP): Consolidated Guideline [~~1~~ ***2***].
Sections 2.0, 2.1, 5.2, 6.1, and 9.3.5	Days ~~93~~ ***94***, ~~95~~ ***96***, ~~98~~ ***99***, ~~101~~ ***102***, and ~~104~~ ***105***.
Section 9.1.7	Days ~~93~~***94***, ~~95~~***96***, and ~~104~~ ***105***.
Section 9.1.10	Days ~~95~~***96***, ~~98~~ ***99***, ~~101~~ ***102***, and ~~104~~ ***105***.
Section 9.1.4	Physical examinations must be performed by a qualified health professional in accordance with local regulations and as listed within the Site Responsibility Delegation Log. A complete physical examination will be performed ***at Screening (if applicable) and*** prior to vaccination on Day 1 (M0) and Day 90 (M3). Measurement of height is only required at ***Screening or*** Day 1 (M0)***, as applicable***.
Section 9.1.6	***9.1.6 Screening*** ***A blood sample (2.5 mL) will be collected at the screening visit (up to 35 days [5 weeks] prior to Day 1 [M0]). A screening visit is only applicable if serological testing with regard to the inclusion criteria (see Section 7.1) is performed within the scope of this trial. All blood samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guideline provided to the trial site.*** Note: The renumbering of subsequent heading 3 sections following addition of this section is not documented.
Section 9.1.11	For female subjects of childbearing potential, serum or urine pregnancy testing will be performed ***at Screening (if applicable) and*** prior to each trial dose administration (Day 1 [M0] and Day 90 [M3]).

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Section	Description of change
Section 9.3.1	***Section 9.3.1 Procedures at the Screening Visit (up to 35 days [5 weeks] prior to Day 1 [M0]).*** ***Note: A screening visit is only applicable if serological testing with regard to the inclusion criteria (see Section 7.1) is performed within the scope of this trial.*** ***1. Before performing any trial procedure, the signed informed consent form needs to be obtained. Refer to Section 9.1.1.*** ***2. Check inclusion and exclusion criteria. Refer to Section 7.1 and Section 7.2, respectively.*** ***3. Collect demographic data, medical history, and prior medication/vaccination. Refer to Section 9.1.2.*** ***4. Collect concomitant medications/vaccinations. Refer to Section 9.1.2.*** ***5. Perform a complete physical examination. Refer to Section 9.1.4.*** ***6. Perform pregnancy testing (serum or urine) for females of childbearing potential. Refer to Section 9.1.11*** ***7. Collect blood sample. Refer to Section 9.1.6.*** ***Blood should be taken from the subject using an aseptic venipuncture technique. Refer to the detailed collection and handling procedures outlined in the Procedures Manual.*** ***The site should schedule the next trial visit with the subject.*** ***The subject will receive a written reminder of the next trial visit.*** Note: The renumbering of subsequent heading 3 sections following addition of this section is not documented.
Section 9.3.2	1. Before performing any trial procedure, the signed informed consent form needs to be obtained (Day 1 [M0] ***unless obtained at Screening***). Refer to Section 9.1.1.
Section 9.4	In this trial, specimens will be collected for ***serological testing as described in Section 9.1.6,*** immune response testing as described in Section ~~9.1.6~~ ***9.1.7***. and Section ~~9.1.7~~ ***9.1.8***, and for clinical safety as described in Section ~~9.1.9~~ ***9.1.10***.
Section 15.4.2	~~Primary Completion of~~ Trial ***completion*** corresponds to the date on which the final subject was examined or received an intervention for the purposes of final collection of data ***(Last Subject Last Visit)*** ~~for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was terminated~~.

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Section	Description of change
Section 16.0	10. ~~Sanofi Pasteur, 2011. http://www.dengue.info [Accessed 22 October 2017]~~ ***Wichmann O, Vannice K, Asturias EJ, de Albuquerque Luna EJ, Longini I, Lopez AL, et al. Live-attenuated tetravalent dengue vaccines: The needs and challenges of post-licensure evaluation of vaccine safety and effectiveness. Vaccine. 2017. 35(42):5535-42.*** 12. ~~Sanofi Pasteur, 2017. http://mediaroom.sanofi.com/sanofi-updates-information-on-dengue-vaccine [Accessed 12 March 2018]~~ ***Sridhar S, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T, et al. Effect of dengue serostatus on dengue vaccine safety and efficacy. N Engl J Med. 2018. 379(4):327-40.***
Section 16.0	***13. World Health Organization. Weekly Epidemiological Record. 2018. 93:329-44. Available at http://www.who.int/wer [Accessed 22 October 2018].*** ***16. Huang CY, Butrapet S, Pierro DJ, Chang GJ, Hunt AR, Bhamarapravati N, et al. Chimeric dengue type 2 (vaccine strain PDK-53)/dengue type 1 virus as a potential candidate dengue type 1 virus vaccine. J Virol. 2000;74(7):3020-8.*** ~~18~~ ***20*** WHO Drug Dictionary. ~~http://www.wow.com/wiki/WHO_Drug_Dictionary [Accessed 22 November 2017]~~ ***Available at http://www.who.int/medicines/ publications/druginformation/en/ [Accessed 23 January 2019]***

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1.3 Protocol Amendment Summary of Changes

This document describes the changes in reference to the Protocol incorporating Amendment No. 2.

1.3.1 Amendment History

Date	Amendment Number	Amendment Type	Region
02 May 2018	Initial Protocol	Not applicable	Local (United States of America)
23 January 2019	1	Non-substantial	Local (United States of America)
06 December 2019	2	Substantial	Local (United States of America)

1.3.2 Summary of Changes

Amendment to Protocol Version 2.0 dated 23 January 2019

Rationale for the Amendment:

This protocol has been amended because incidence estimates of dengue among travelers indicates that although the incidence rate is variable (due to the multifactorial nature of dengue transmission in endemic countries), overall, the risk of dengue transmission among travelers is considered to be low (1-4). As a result, the criterion #22 that excludes subjects from participating in the trial based on planned travel to dengue endemic countries during the conduction of the trial has been removed. Removal of this exclusion criteria is expected to address, at least to some extent, the slow enrollment rate experienced in the trial.

In addition, the screening window for subjects screened within the scope of this trial has been increased to 70 days (10 weeks) to allow enrollment of subjects falling outside the previous defined screening window of 35 days (5 weeks).

1. Baaten GG, Sonder GJ, Zaaijer HL, van Gool T, Kint JA, van den Hoek A. Travel-related dengue virus infection, The Netherlands, 2006-2007. Emerg Infect Dis 2011;17(5):821-8.
2. Cobelens FG, Groen J, Osterhaus AD, Leentvaar-Kuipers A, Wertheim-van Dillen PM, Kager PA. Trop Med Int Health 2002;7(4):331-8.
3. Potasman I, Srugo I, Schwartz E. Dengue seroconversion among Israeli travelers to tropical countries. Emerg Infect Dis 1999;5(6):824-7.
4. Ratnam I, Black J, Leder K, Biggs BA, Matchett E, Padiglione A, et al. Incidence and seroprevalence of dengue virus infections in Australian travellers to Asia. Eur J Clin Microbiol Infect Dis 2012;31(6):1203-10.

Other modifications:

- Administrative change
- Update of literature list
- Minor editorial changes are included for consistency purposes only.

New text is shown in bold italics and any deleted text is marked using strikethrough.

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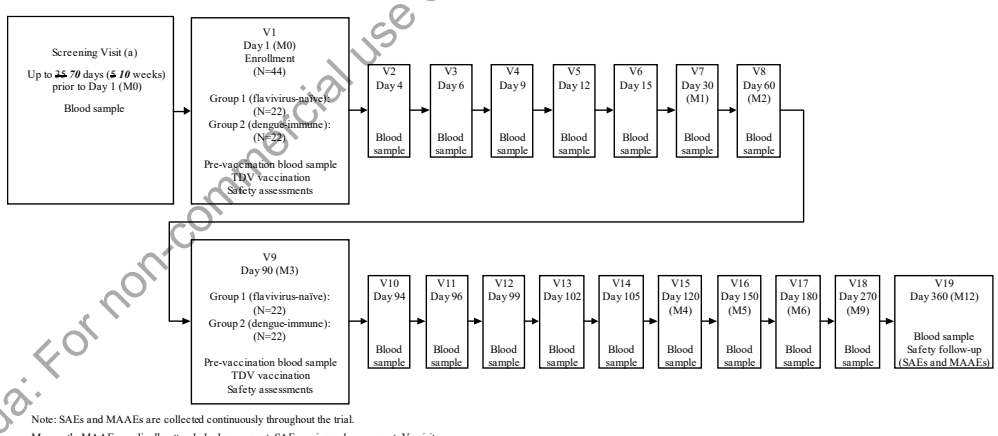
Section	Description of change
Title page	Date: ~~23 January 2019~~ ***06 December 2019*** Version: Version ~~2.0~~ ***3.0*** (supersedes Version ~~1.0~~ ***2.0***)
1.2	[redacted], ~~PhD~~ [redacted], ***MSc*** [redacted] Vaccines Statistics Global Statistics Takeda Pharmaceuticals International AG
Sections 2.0, 6.1	Subjects will be enrolled in 2 trial groups based on results from serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to ~~35~~ ***70*** days [~~5~~ ***10*** weeks] prior to Day 1[M0]): All subjects will be followed-up for 9 months post second vaccination so the trial duration will be approximately 360 days (12 months) or ~~13~~ ***14.5*** months for each subject depending on whether serological testing with regard to the inclusion criteria for the trial is performed outside or within the scope of this trial, respectively.
Sections 2.0, 6.1, Figure 2.a, Figure 6.a	– Group 2: approximately 22 dengue-immune subjects (ie, ~~approximately 11~~ subjects with serology consistent with primary infection by DENV-1 and ~~approximately 11~~ subjects with serology consistent with primary infection by DENV-3).  Note: SAEs and MAAEs are collected continuously throughout the trial. M=month, MAAE=medically attended adverse event, SAE=serious adverse event, V=visit (a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.

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<tr><td>Sections 2.0 and 7.2</td><td>~~22. Planned travel (during the trial conduct) to any area endemic for dengue.~~</td></tr>
<tr><td>Section 2.1, Table 2.a</td><td>Table 2.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 [M12])
<table>
<tr><th></th><th>Screening visit [a]</th><th>V 1</th><th>V 2</th><th>V 3</th><th>V 4</th><th>V 5</th><th>V 6</th><th>V 7</th><th>V 8</th><th>V 9</th><th>V 10</th><th>V 11</th><th>V 12</th><th>V 13</th><th>V 14</th><th>V 15</th><th>V 16</th><th>V 17</th><th>V 18</th><th>V 19</th></tr>
<tr><td>Visit window (days)</td><td>Up to ~~35~~ ***70*** days (~~5~~ ***10*** weeks) prior to D1 (M0)</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Record travel history and planned travel to any country</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td></tr>
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Diary card[~~e~~ ***p***]
AEs leading to trial vaccine withdrawal or trial discontinuation, SAEs, MAAEs[~~f~~ ***q***]</td></tr>
<tr><td>Sections 2.0 and 6.3</td><td>The trial duration for each subject will be at least approximately 360 days (12 months) including vaccination (Day 1 [M0] and Day 90 [M3]) and follow-up through Day 360 (M12). This duration will be approximately ~~13~~ ***14.5*** months if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial (ie, up to ~~35~~ ***70*** days [~~5~~ ***10*** weeks] prior to Day 1 [M0]).</td></tr>
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Section	Description of change
Section 9.1.6	A blood sample (2.5 mL) will be collected at the screening visit (up to ~~35~~ ***70*** days [~~5~~ ***10*** weeks] prior to Day 1 [M0)]). A screening visit is only applicable if serological testing with regard to the inclusion criteria (see Section 7.1) is performed within the scope of this trial. All blood samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guideline provided to the trial site.
Section 9.3.1	9.3.1 Procedures at the Screening Visit (up to ~~35~~ ***70*** days [~~5~~ ***10*** weeks] prior to Day 1 [M0]). ***Record travel history and planned travel to any country.***
Sections 9.3.2 and 9.3.5	***Record travel history and planned travel to any country.***
Section 16.0	2. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH harmonised guideline. Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2). Available at ~~http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2_Step_4_2016_109.pdf~~. ***https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf*** [Accessed ~~23 January~~ ***06 December*** 2019]. 6. World Health Organization, 2018. Dengue and severe dengue. Fact Sheet. Available at ~~http://www.who.int/mediacentre/factsheets/fs117/en/~~ ***https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue*** [Accessed ~~23 January~~ ***06 December*** 2019]. 7. World Health Organization, 1997. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control, 2nd Edition. Geneva. Available at https://www.who.int/csr/resources/publications/dengue/Denguepublication/en/ [Accessed ~~23 January~~ ***06 December*** 2019]. 8. World Health Organization, 2009. Dengue guidelines for diagnosis, treatment, prevention and control. Available at http://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf. [Accessed ~~23 January~~ ***06 December*** 2019]. 13. World Health Organization. Weekly Epidemiological Record. 2018. 93:329-44. Available at http://www.who.int/wer [Accessed ~~23 January~~ ***06 December*** 2019]. 17. Policy and communication bulletin – The clinical center. Guidelines for limits of blood drawn for research purposes in the clinical center. Manual transmittal sheet, no. M95-9 (rev.), 2009. Available at https://irb.research.chop.edu/.../files/documents/g_nih_blooddraws [Accessed ~~23 January~~ ***06 December*** 2019]. 20. WHO Drug Dictionary. Available at http://www.who.int/medicines/publications/druginformation/en/ [Accessed ~~23 January~~ ***06 December*** 2019].

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1.3 Protocol Amendment Summary of Changes

This document describes the changes in reference to the Protocol incorporating Amendment No. 3.

1.3.1 Amendment History

Date	Amendment Number	Amendment Type	Region
02 May 2018	Initial Protocol	Not applicable	Local (United States of America)
23 January 2019	1	Non-substantial	Local (United States of America)
06 December 2019	2	Substantial	Local (United States of America)
11 March 2020	3	Substantial	Local (United States of America)

1.3.2 Summary of Changes

1. Amendment to Protocol Version 3.0 dated 06 December 2019

2. Rationale for the Amendment:

3. This protocol amendment is due to the slow enrollment rate experienced in the trial for Group 2 (DENV-1 or DENV-3 positive subjects) while complete enrollment of the planned number of subjects in Group 1 (flavivirus-negative subjects) has been achieved (n=22). Group 2 would create a subject population potentially eligible for inclusion in a future DENV-1/DENV-3 human challenge trial. Since Group 1 is considered a key group for the characterization of immune responses to Takeda's Tetravalent Dengue Vaccine Candidate (TDV) (primary and secondary trial objectives), it was decided to stop the recruitment of subjects in Group 2. As a result, the criterion that excludes subjects from participating in the trial based on planned travel to dengue endemic countries during the conduct of the trial which was removed from Protocol Version 3.0 dated 06 December 2019 is reinstated since 1) removal of this criterion was previously done to facilitate enrollment of subjects in Group 2, and 2) travel to dengue endemic regions is undesirable for subjects in Group 1 and Group 2. As the criterion of authorized travel to dengue endemic countries is no longer included, recording of the travel history and planned trips to any country is no longer necessary.
4. Protocol Version 3.0 dated 06 December 2019 has not been submitted to any Institutional Review Boards.
5. Other modifications:

– Administrative change
– Correction in the unit for the non-structural protein 1 (NS1) antibody test result.
– Removal of reference to the Procedures Manual as it is not applicable for the trial.
– A minor editorial change is included for consistency purposes only.
– Update of literature list

6. New text is shown in bold italics and any deleted text is marked using strikethrough.

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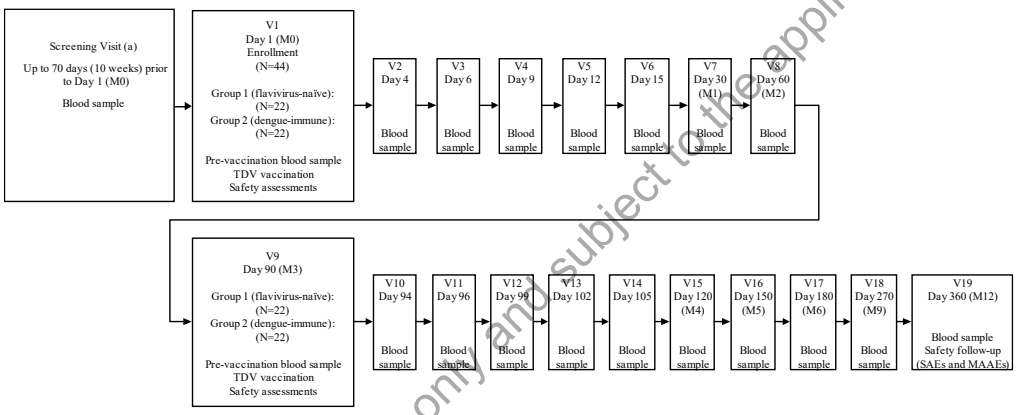
Section	Description of change
Title page	Date: ~~06 December 2019~~ ***11 March 2020*** Version: Version ~~3.0~~ ***4.0*** (supersedes Version ~~2.0~~ ***3.0***)
Section 1.2	[redacted]~~, MPH~~ [redacted] [redacted] Vaccine Business Unit Takeda Vaccines, Inc
Sections 2.0, 6.1, Figure 2.a, Figure 6.a	***Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.***
	 Note: SAEs and MAAEs are collected continuously throughout the trial. ***Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.*** M=month, MAAE=medically attended adverse event, SAE=serious adverse event, V=visit (a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.
Sections 2.0 and 7.2	***7. 22. Planned travel (during the trial conduct) to any area endemic for dengue.***
Sections 2.1, 9.3.1, 9.3.2 and 9.3.5, Table 2.a	~~Record travel history and planned travel to any country~~
Sections 2.0 and 5.2.3	• Average ~~EC_{50}~~ ***concentration (relative units/mL)*** of anti-dengue NS1 antibodies for each of the 4 dengue serotypes measured by ELISA using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
Section 9.1.5	~~Body temperature measurement will be described in the Procedures Manual~~.
Sections 9.3.1 9.3.2, and 9.3.5	~~Refer to the detailed collection and handling procedures outlined in the Procedures Manual.~~

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Section 13.1	Data reviews will be conducted prior to the planned interim analysis and final analysis. ~~This~~ ***These*** review***s*** will assess the accuracy and completeness of the trial database, subject evaluability, and appropriateness of the planned statistical methods.
Section 16.0	2. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH harmonised guideline. Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2). Available at https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf [Accessed ~~06 December 2019~~ ***11 March 2020***]. 8. 6. World Health Organization, 2018. Dengue and severe dengue. Fact Sheet. Available at https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue [Accessed ~~06 December 2019~~ ***11 March 2020***]. 7. World Health Organization, 1997. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control, 2nd Edition. Geneva. Available at https://www.who.int/csr/resources/publications/dengue/Denguepublication/en/ [Accessed ~~06 December 2019~~ ***11 March 2020***]. 8. World Health Organization, 2009. Dengue guidelines for diagnosis, treatment, prevention and control. Available at http://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf. [Accessed ~~06 December 2019~~ ***11 March 2020***]. 13. World Health Organization. Weekly Epidemiological Record. 2018. 93:329-44. Available at http://www.who.int/wer [Accessed ~~06 December 2019~~ ***11 March 2020***]. 9. 17. Policy and communication bulletin – The clinical center. Guidelines for limits of blood drawn for research purposes in the clinical center. Manual transmittal sheet, no. M95-9 (rev.), 2009. Available at https://irb.research.chop.edu/.../files/documents/g_nih_blooddraws [Accessed ~~06 December 2019~~ ***11 March 2020***]. 10. 20. WHO Drug Dictionary. Available at http://www.who.int/medicines/publications/druginformation/en/ [Accessed ~~06 December 2019~~ ***11 March 2020***].

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An Open-Label, Phase 2 Trial to Investigate the Humoral and Cell-Mediated Immune Responses and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Flavivirus-Naïve and Dengue-Immune Healthy Adults

Immunogenicity and Safety of TDV in Flavivirus-Naïve and Dengue-Immune Adults

Sponsor:	Takeda Vaccines, Inc. 40 Landsdowne Street Cambridge, MA 02139 USA
Trial Identifier:	DEN-210
IND Number:	014292 **EudraCT Number:** Not Applicable
Trial Vaccine Name:	Takeda's tetravalent dengue vaccine candidate (TDV) comprised of a molecularly characterized, attenuated dengue serotype 2 strain (TDV-2), a dengue serotypes 2/1 chimeric strain (TDV-1), a dengue serotypes 2/3 chimeric strain (TDV-3), and a dengue serotypes 2/4 chimeric strain (TDV-4).
Takeda Approval Date:	18 December 2020
Version:	Version 5.0 (supersedes Version 4.0)

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1.0 ADMINISTRATIVE INFORMATION

1.1 Contacts

Issue	Contact
Serious adverse event and pregnancy reporting	IQVIA Integrated Safety Management Lifecycle Safety Fax: +1 919 800 0122 Hotline: +91 80 71311011 E-mail: TakedaDensafety@Quintiles.com

1.2 Approval

REPRESENTATIVES OF TAKEDA

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

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

SIGNATURES

{See appended signature page}

[redacted], PharmD, DPhil Date
[redacted], Clinical Development
Vaccine Business Unit
Takeda Pharmaceuticals International AG

{See appended signature page}

[redacted], PhD, MSc PV Date
[redacted]
Takeda Pharmaceuticals International AG

{See appended signature page}

[redacted], MSc Date
[redacted]
Vaccines Statistics
Global Statistics
Takeda Pharmaceuticals International AG

{See appended signature page}

[redacted] Date
[redacted]
Vaccine Business Unit
Takeda Vaccines, Inc.

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INVESTIGATOR AGREEMENT

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

- The ethical principles that have their origin in the Declaration of Helsinki [1].
- ICH E6 GCP: Consolidated Guideline [2].
- All applicable laws and regulations, including, but not limited to those related to data privacy and clinical trial disclosure.
- Regulatory requirements for reporting Serious Adverse Events (SAEs) defined in Section 10.4.4 of this protocol.
- Terms outlined in the Clinical Trial Site Agreement.
- Appendix A – Responsibilities of the Investigator.

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

Signature of Investigator	Date

Investigator Name (print or type)

Investigator's Title

Location of Facility (City, State)

Location of Facility (Country)

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1.3 Protocol Amendment Summary of Changes

This document describes the changes in reference to the Protocol incorporating Amendment No. 4.

1.3.1 Amendment History

Date	Amendment Number	Amendment Type	Region
02 May 2018	Initial Protocol	Not applicable	Local (United States of America)
23 January 2019	1	Non-substantial	Local (United States of America)
06 December 2019	2	Substantial	Local (United States of America)
11 March 2020	3	Substantial	Local (United States of America)
18 December 2020	4	Substantial	Local (United States of America)

1.3.2 Summary of Changes

Amendment to Protocol Version 4.0 dated 11 March 2020

Rationale for the Amendment:

This protocol amendment is to remove the planned interim analysis due to the short time period between the projected dates for the database lock for the interim analysis (March 2021) and final analysis (August 2021). The interim analysis would have provided safety and immunogenicity data when all subjects have completed the Day 120 (M4) visit. Given the anticipated timing of the database lock for both analyses, it is considered that the interim analysis is no longer needed.

Other modifications:

- Addition of a clarification that, due to the COVID-19 pandemic, alternative monitoring approaches may be used.
- Addition of a clarification that some of the test results may not be available prior to database lock because trial laboratory procedures are not carried out during the COVID-19 pandemic.
- Following modifications were made for alignment with protocol template Version 4.0:
  - Update in responsibilities of the Signatory Investigator.
  - Addition of trial risk management.
- Administrative change
- Few minor editorial changes.

New text is shown in bold italics and any deleted text is marked using strikethrough.

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Section	Description of change
Title page	Takeda Approval Date: ~~11 March~~ ***18 December*** 2020 Version: Version ~~4.0~~ ***5.0*** (supersedes Version ~~3.0~~ ***4.0***)
1.2	[redacted]~~, MD~~ [redacted]~~, Clinical Development~~ ~~Vaccine Business Unit~~ ~~Takeda Pharmaceuticals USA, Inc.~~ [redacted]***, PharmD, DPhil*** [redacted]***, Clinical Development Vaccine Business Unit*** ***Takeda Pharmaceuticals International AG***
1.3	This document describes the changes in reference to the Protocol incorporating Amendment No. ~~3~~ ***4***.
2.0	**Interim Analysis:** ~~An interim analysis of immunogenicity and safety data is planned when all subjects have completed the Day 120 (M4) visit.~~ ***No interim analysis is planned.***
2.1	**Table 2.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 [M12])** ***Note: When a site visit cannot be carried out due to the COVID-19 pandemic, telephone contacts will be made for subjects who are still under monitoring for safety reporting.***
3.2	~~The sponsor will select a Signatory Principal Investigator from the investigators who participate in the trial~~. Selection criteria for ~~this~~ ***the Principal i****I*nvestigator will include significant knowledge of the trial protocol, the investigational vaccine, their expertise in the therapeutic area and the conduct of clinical research as well as trial participation. ***Takeda will select a Signatory Investigator from the investigators who participate in the trial.*** The Signatory ~~Principal~~ Investigator will be required to review and sign the clinical ***protocol. The Signatory Investigator will also be required to review and sign the c****Clinical* ~~s~~***S***tudy ~~r~~***R***eport ***(CSR)*** and by doing so agrees that it accurately describes the results of the trial.
3.3	***CSR*** — ***Clinical Study Report*** **MHRA** — ***Medicines and*** Healthcare Products Regulatory Agency of the United Kingdom ***QTL*** — ***Quality Tolerance Limits***
6.1	~~Refer to Section 13.2 for the planned interim analysis.~~
9.3.7	~~Not applicable.~~ ***When a site visit cannot be carried out due to the COVID-19 pandemic, telephone contacts will be made for subjects who are still under monitoring for safety reporting. Refer also to Section 14.1.***
12.1	***When a site visit cannot be carried out due to the COVID-19 pandemic, telephone contacts will be made for subjects for whom monitoring for safety reporting is still ongoing. Refer also to Section 14.1.***

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Section	Description of change
13.1	A Statistical Analysis Plan (SAP) will be prepared and finalized prior to the ~~planned interim analysis~~ ***database lock***. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all trial objectives. Data reviews will be conducted prior to the ~~planned interim analysis and final analysis~~ ***database lock***. These reviews will assess the accuracy and completeness of the trial database~~,~~ ***and*** subject evaluability~~, and appropriateness of the planned statistical methods~~. ***As trial laboratory procedures are not carried out during the COVID-19 pandemic there is a possibility that not all pre-defined test results will be available prior to database lock. Thus, there is a risk for database unlock/relock and additional data may be added in CSR amendments or addenda to the final CSR.***
13.2	~~An interim analysis of immunogenicity and safety data is planned when all subjects have completed the Day 120 (M4) visit.~~ ***No interim analysis is planned.***
14.0	**14.1 Trial-Site Monitoring Visits** ***In the event a monitor cannot visit the site in a timely manner due to the COVID-19 pandemic, alternative monitoring approaches such as remote source data verification (SDV) or telephone contact may be used to ensure data quality and integrity and maintain subject safety. Alternative monitoring approaches should be used only where allowed by the local Health Authority and when approved by the IRB/IEC. During remote monitoring, the monitor should focus on trial activities that are essential to the safety of trial subjects and/or data reliability.*** ***14.4 Trial Risk Management*** ***The ICH E6 addendum (R2) guidance encourages a risk-based approach to the management of clinical trials and includes requirements for risk control and risk reporting. Takeda or designee established Quality Tolerance Limits (QTL) taking into consideration the medical and statistical characteristics of the variables and the statistical design of the trial. This process was performed according to Takeda internal procedures.*** ***At the end of the trial, the quality management approach implemented will be described in the CSR. If applicable, the CSR will summarize important deviations from the predefined QTL and the remedial actions taken***.

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Section	Description of change
16.0	2. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH harmonised guideline. Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2). Available at https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf. [Accessed ~~11 March~~ ***18 December*** 2020]. 6. World Health Organization, 2018. Dengue and severe dengue. Fact Sheet. Available at https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue [Accessed ~~11 March~~ ***18 December*** 2020]. 7. World Health Organization, 1997. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control, 2nd Edition. Geneva. Available at https://www.who.int/csr/resources/publications/dengue/Denguepublication/en/ [Accessed ~~11 March~~ ***18 December*** 2020]. 8. World Health Organization, 2009. Dengue guidelines for diagnosis, treatment, prevention and control. Available at http://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf. [Accessed ~~11 March~~ ***18 December*** 2020]. 13. World Health Organization. Weekly Epidemiological Record. 2018. 93:329-44. Available at http://www.who.int/wer [Accessed ~~11 March~~ ***18 December*** 2020]. 17. Policy and communication bulletin – The clinical center. Guidelines for limits of blood drawn for research purposes in the clinical center. Manual transmittal sheet, no. M95-9 (rev.), 2009. Available at https://irb.research.chop.edu/.../files/documents/g_nih_blooddraws [Accessed ~~11 March~~ ***18 December*** 2020]. 20. WHO Drug Dictionary. Available at http://www.who.int/medicines/publications/druginformation/en/ [Accessed ~~11 March~~ ***18 December*** 2020].
Appendix A	13. Review and provide a signature as approval of the content of the ~~clinical study report~~ ***CSR***

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LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

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2.0 TRIAL SUMMARY

Name of Sponsor: Takeda Vaccines, Inc. 40 Landsdowne Street Cambridge, MA 02139 USA		**Product Name:** Takeda's Tetravalent Dengue Vaccine Candidate (TDV)
Trial Title: An Open-Label, Phase 2 Trial to Investigate the Humoral and Cell-Mediated Immune Responses and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Flavivirus-Naïve and Dengue-Immune Healthy Adults		
IND No.: 014292		**EudraCT No.:** Not applicable
Trial Identifier: DEN-210	**Phase:** 2	**Blinding Schema:** Open Label
Indication: Prevention of dengue fever of any severity due to any serotype.		

Background and Rationale:

Dengue fever is caused by infection with the wild type dengue virus (DENV), a ribonucleic acid virus that occurs as 4 recognized serotypes, DENV-1, DENV-2, DENV-3, or DENV-4. These 4 DENVs are transmitted to humans by mosquitoes (primarily *Aedes aegypti*), and are endemic in Asia, Central and South America, the Caribbean, the Pacific Islands, and parts of Africa. There are an estimated 390 million dengue infections per year worldwide, which is close to 4 times the previous World Health Organization (WHO) estimate of 50 to 100 million cases. Every year, around 500,000 cases of Dengue Hemorrhagic Fever (DHF) require hospitalization with an estimated death rate of 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection.

Dengue fever is clinically defined as an acute febrile illness with 2 or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leukopenia, and occurrence at the same location and time as other confirmed cases of dengue fever. The most severe forms of dengue infection – DHF and Dengue Shock Syndrome (DSS) – are life threatening. Primary infection with any one of the 4 dengue serotypes is thought to result in life-long protection from re-infection by the same serotype but does not protect against a secondary infection by one of the other 3 dengue serotypes which may lead to an increased risk of severe disease (DHF/DSS).

Treatment of dengue fever is based solely on medical management of signs and symptoms, with fluid replacement required for hemorrhagic or shock cases. An antiviral therapy for DENV infection is not available at this time. Preventive measures that rely on mosquito control and individual protection are of limited efficacy, complex to implement and questionable in terms of cost-effectiveness. There is a great unmet global public health need for a safe and effective vaccine to reduce the morbidity and mortality associated with dengue disease. Vaccine development has focused on tetravalent vaccines that provide protection against all 4 dengue serotypes simultaneously since all 4 dengue serotypes commonly co-circulate in endemic areas. A first dengue vaccine (Chimeric Yellow fever virus Dengue virus-Tetravalent Dengue Vaccine [CYD-TDV]) has been approved (year 2015) in some countries in Asia and Latin America. Initial findings showed that vaccine efficacy was different between serotypes and depended on dengue pre-exposure status. Additionally, recent analyses found that people who had not been infected by dengue virus before vaccination had a higher risk of getting severe disease when they were infected with dengue virus after vaccination with CYD-TDV. Hence, there is a continued unmet public health need for safer and more efficacious dengue vaccines.

Takeda's Tetravalent Dengue Vaccine Candidate (TDV) - Background:

Takeda's TDV consists of 1 molecularly characterized, attenuated dengue serotype 2 virus strain and 3 chimeric recombinant dengue virus strains expressing surface antigens corresponding to DENV serotypes 1– 4. The dengue serotype 2 strain (TDV-2) is based upon the attenuated laboratory-derived DENV-2 virus strain, originally isolated at Mahidol University, Bangkok, Thailand and generated by 53 serial passages in Primary Dog Kidney (PDK) cells (DENV-2 PDK-53). The chimeric, attenuated vaccine strains for dengue serotypes 1, 3 and 4 were engineered by substituting the structural genes, pre-Membrane (prM) and Envelope (E), of TDV-2 with the prM and E genes of the DENV virus strains, DENV-1 16007, DENV- 3 16562 or DENV-4 1036 virus, respectively. Thus, TDV is comprised of 4 dengue virus strains: a molecularly characterized, attenuated dengue serotype 2 strain (TDV-2), a dengue serotypes 2/1 chimeric

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strain (TDV-1), a dengue serotypes 2/3 chimeric strain (TDV-3), and a dengue serotypes 2/4 chimeric strain (TDV-4).

Nonclinical studies carried out in mice and nonhuman primates have demonstrated an acceptable safety, immunogenicity, and efficacy profile of Takeda's TDV. Additionally, data from completed phase 1 and phase 2 clinical trials in humans have shown satisfactory reactogenicity, safety and immunogenicity profiles of Takeda's TDV in healthy adults in nonendemic areas as well as in healthy adults and children in endemic areas in Asia and Latin America. Ongoing and completed phase 2 clinical trials have enabled the selection of a final TDV dose (lyophilized formulation), and a 2-dose vaccination regimen (2 single doses) administered 3 months (ie, 90 days) apart by subcutaneous (SC) injection for use in the ongoing pivotal program.

The current Investigator's Brochure contains additional product information and a more detailed review of nonclinical studies and clinical trials.

Rationale for the Proposed Trial:

The purpose of this trial is to characterize the magnitude, quality, and evolution over time of humoral and cell-mediated immune responses to TDV when administered as a 2-dose vaccination regimen (2 single doses 3 months [ie, 90 days] apart). Furthermore, this trial aims to characterize the relationships between TDV- specific innate, humoral and cellular immune responses. This characterization will aid in the understanding of vaccine efficacy and in the identification of potential determinants for protection against all 4 dengue serotypes in the ongoing phase 3 TDV clinical program. This trial will be conducted at trial centers in the United States of America involving healthy adults who are either flavivirus-naïve or seropositive for DENV-1 or DENV-3 (ie, serology consistent with primary infection with either DENV-1 or DENV-3). The serological status of subjects will either have been determined by the trial center prior to and outside the scope of this trial or will be determined through screening within the scope of this trial.

Although certain parameters of TDV-specific humoral and cell-mediated immune responses have been evaluated to some extent in phase 1 and other phase 2 clinical trials with TDV, the present trial is the only one that will allow a comprehensive analysis of TDV-specific humoral, T cell-mediated, B cell-mediated, innate, and early immune responses. In particular, these responses following 2 doses of TDV in both flavivirus-naïve and dengue-immune adults have not been evaluated yet in any other single trial in the TDV clinical program. Furthermore, conduct of the present trial would create a subject population, potentially eligible for inclusion in a future DENV-1/DENV-3 human challenge trial. Such a trial may be considered at a later date by the sponsor if deemed appropriate to further guide TDV development.

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

Trial Design:

This is an open-label, phase 2 trial in 44 healthy adult subjects aged 18 to 60 years (inclusive) to investigate the immunogenicity and safety of SC administration of a 2-dose regimen of TDV.

Subjects will be enrolled in 2 trial groups based on results from serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 70 days [10 weeks] prior to Day 1 [Month 0 (M0)]):

- Group 1: approximately 22 flavivirus-naïve subjects.
- Group 2: approximately 22 dengue-immune subjects (ie, subjects with serology consistent with primary infection by DENV-1 and subjects with serology consistent with primary infection by DENV-3).

Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.

TDV will be administered on Day 1 (M0) and on Day 90 (Month 3 [M3]) in both Groups 1 and 2.

The blood sampling schedule is summarized in Figure 2.a. In compliance with the National Institutes of Health guidance, the total amount of blood that will be drawn from trial participants over an 8-week period will not exceed 10.5 mL/kg or 550 mL (whichever is smaller).

All subjects will be followed-up for 9 months post second vaccination so the trial duration will be approximately 360 days (12 months) or 14.5 months for each subject depending on whether serological testing with regard to the inclusion criteria for the trial is performed outside or within the scope of this trial, respectively. The trial schedule is presented in Figure 2.a.

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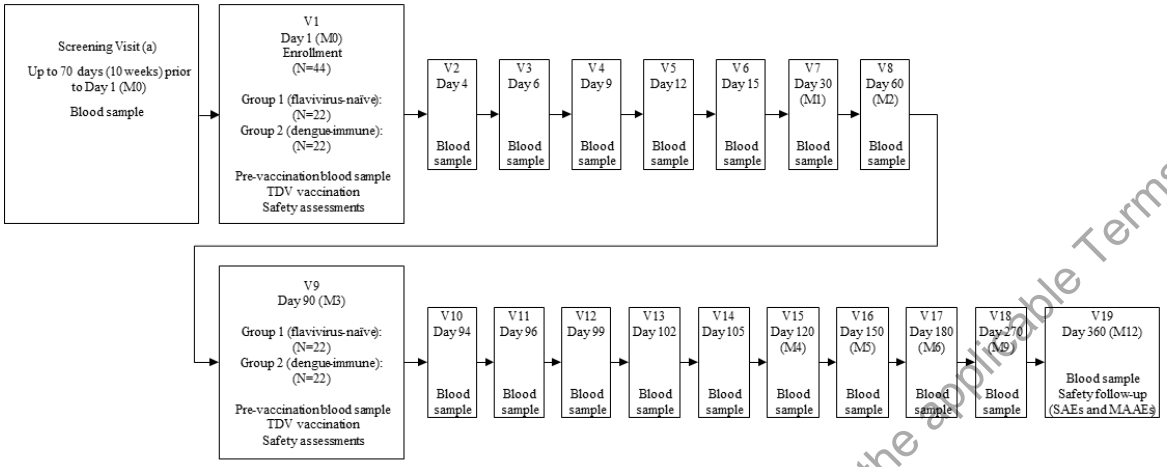
Figure 2.a Schematic of DEN-210 Trial Design



Note: SAEs and MAAEs are collected continuously throughout the trial.
Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.
M=month, MAAE=medically attended adverse event, SAE=serious adverse event, V=visit
(a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.

Immunogenicity evaluations:

- Dengue neutralizing antibodies will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (Month 1 [M1]), and 60 (Month 2 [M2]); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (Month 4 [M4]), 150 (Month 5 [M5]), 180 (Month 6 [M6]), 270 (Month 9 [M9]), and 360 (Month 12 [M12]).
- T cell-mediated immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- B cell-mediated immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Day 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 180 (M6), 270 (M9), and 360 (M12).
- T cell epitopes will be mapped using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Anti-dengue non-structural protein 1 (NS1) antibodies will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- The innate immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 4 and 6; pre second vaccination (Day 90 [M3]), and on Days 94 and 96.

Safety evaluations:

- Diary cards (paper or electronic) will be distributed for the recording of:
  - Solicited Adverse Events (AEs):
    - Solicited local (injection site) reactions for 7 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 6 days). These will include: injection site pain, injection site erythema, and injection site swelling.
    - Solicited systemic events for 14 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 13 days). These will include: fever, headache, asthenia, malaise, and myalgia.
  - Unsolicited AEs for 28 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 27 days).

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- Serious Adverse Events (SAEs), Medically Attended Adverse Events (MAAEs), and AEs leading to trial vaccine withdrawal or trial discontinuation will be collected for the trial duration. MAAEs are defined as AEs leading to an unscheduled medical visit to or by a healthcare professional including visits to an emergency department, but not fulfilling seriousness criteria.

Vaccine viremia evaluation:

- Vaccine viremia will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 6, 9, 12, 15, and 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 96, 99, 102, 105, and 120 (M4).

Data collection will be by electronic Case Report Form (eCRF).

Primary Objective:

- To assess the neutralizing antibody response (Geometric Mean Titers [GMT]) against each dengue serotype post-vaccination (Microneutralization Test 50% [MNT_{50}]).

Secondary Objectives:

Immunogenicity

- To assess the magnitude (Interferon-gamma Enzyme-Linked Immunospot [IFN-γ ELISpot]) and polyfunctionality (Intracellular Cytokine Staining [ICS]) of the T cell-mediated immune response post-vaccination.
- To assess vaccine viremia post-vaccination and the integrity of main attenuation mutations.

Safety

- To describe the safety of 2 doses of TDV in healthy subjects aged 18 to 60 years (inclusive).

Exploratory Objectives:

- To assess the neutralizing antibody response against each dengue serotype post-vaccination (dengue Reporter Virus Particle [RVP] test).
- To assess the B cell-mediated immune response post-vaccination (Quad-color FluoroSpot).
- To assess the anti-dengue NS1 antibody response post-vaccination (Enzyme-Linked ImmunoSorbent Assay [ELISA]).
- To map the epitopes of the dengue antibodies post-vaccination (IFN-γ ELISpot response).
- To assess the innate immune response post-vaccination.
- To characterize the relationships between TDV-specific innate, humoral and cellular immune responses on an individual level post-vaccination (MNT_{50}, dengue RVP, Quad color FluoroSpot, NS1 antibody ELISA, IFN- γ ELISpot, ICS, and exploratory assays).
- To characterize the relationships between vaccine viremia (quantitative Reverse Transcription-Polymerase Chain Reaction [qRT-PCR]) and TDV-specific innate, humoral and cellular immune responses on an individual level post-vaccination (MNT_{50}, dengue RVP, Quad color FluoroSpot, NS1 antibody ELISA, IFN-γ ELISpot, ICS, and exploratory assays).

Subject Population:

Healthy Subjects: yes

Age Range: ≥18 to ≤60 years

Planned Number of Subjects: 44

Planned Number of Trial Arms: 2 trial groups (22 subjects per trial group); 2-dose regimen (1 trial vaccine dose on Day 1 [M0] and 1 trial vaccine dose on Day 90 [M3]), SC route.

Estimated Total: 44 enrolled subjects.

Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.

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Key Inclusion Criteria:

1. The subject is aged ≥18 to ≤60 years.
2. Male or female.
3. Subjects who are in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs) and clinical judgment of the investigator.
4. Group 1 only: immunologically naïve to dengue, Zika, Yellow Fever (YF), Japanese Encephalitis (JE), West Nile (WN) (based on negative results for detection of anti-DENV, anti-Zika, anti-YF, anti-JE, anti- WN antibodies) as documented by serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 70 days [10 weeks] prior to Day 1 [M0]).
5. Group 2 only: serology consistent with primary infection with either DENV-1 or DENV-3 (defined as detectable neutralizing antibodies against DENV-1 or DENV-3 only, or titers for DENV-1 or DENV- 3 ≥4-times higher than titers for the 2 other dengue serotypes) as documented by serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 70 days [10 weeks] prior to Day 1 [M0]).
6. The subject signs and dates a written, informed consent and any required privacy authorization prior to the initiation of any trial procedures, after the nature of the trial has been explained according to local regulatory requirements.
7. Subjects who can comply with trial procedures and are available for the duration of follow-up.

Key Exclusion Criteria:

1. Subjects with a clinically active significant infection (as assessed by the investigator) or body temperature ≥38°C (100.4°F) within 3 days of the intended date of vaccination (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration, see below).
2. Subjects with a known hypersensitivity or allergy to any of the trial vaccine components (including excipients).
3. Subjects with behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.
4. Subjects with any history of progressive or severe neurologic disorder, seizure disorder or neuro-inflammatory disease (eg, Guillain-Barré syndrome).
5. Subjects with any illness, or history of any illness that, in the opinion of the investigator, might interfere with the results of the trial or pose additional risk to the subjects due to participation in the trial.
6. Known or suspected impairment/alteration of immune function (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration, see below), including:
   a) Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks and/or ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0) (use of inhaled, intranasal, or topical corticosteroids is allowed).
   b) Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks and/or ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0).
   c) Administration of immunoglobulins and/or any blood products within 3 months prior to Day 1 (M0) or planned administration during the trial.
   d) Receipt of immunostimulants within 60 days prior to Day 1 (M0).
   e) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (M0).
   f) Known Human Immunodeficiency Virus (HIV) infection or HIV-related disease.
   g) Hepatitis C virus infection.
   h) Genetic immunodeficiency.

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7. Abnormalities of splenic or thymic function.
8. Subjects with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
9. Subjects with any serious chronic or progressive disease according to the judgment of the investigator (eg, neoplasm, hematologic malignancies, insulin dependent diabetes; cardiac, renal, or hepatic disease).
10. Subjects with Body Mass Index (BMI) greater than or equal to 35 kg/m^2 (=weight in kg/ height in meters^2).
11. Subjects participating in any clinical trial with another investigational product 30 days prior to Day 1 (M0) or intending to participate in another clinical trial at any time during the conduct of this trial.
12. Subjects who have received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in this trial or who are planning to receive any vaccine within 28 days of trial vaccine administration (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration, see below).
13. Subjects who have used antipyretics and/or analgesic medications within 24 hours prior to vaccination. The reason for their use (prophylaxis versus treatment) must be documented. Trial entry should be delayed to allow for a full 24 hours to have passed since last use of antipyretics and/or analgesic medications (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration, see below).
14. Subjects involved in the trial conduct or their first-degree relatives.
15. Subjects with history of substance or alcohol abuse within the past 2 years.
16. Female subjects who are pregnant or breastfeeding.
17. Female subjects of childbearing potential[1] who are sexually active with men and have not used any of the "acceptable contraceptive methods" [2] for at least 2 months prior to Day 1 (M0).
18. Female subjects of childbearing potential who are sexually active with men and refuse to use an acceptable contraceptive method up to 6 weeks after the last dose of trial vaccine (Day 90 [M3] + 6 weeks). In addition, female subjects must be advised not to donate ova during this period.
19. Any positive or indeterminate pregnancy test.
20. Previous participation in any clinical trial of a dengue candidate vaccine, except for subjects who received placebo in those trials, or previous and planned vaccination (during the trial conduct) against dengue.
21. Planned vaccination (during the trial conduct) against any non-dengue flavivirus (eg, Zika, YF, JE, WN, tick-borne encephalitis, or Murray-Valley encephalitis).
22. Planned travel (during the trial conduct) to any area endemic for dengue.

There may be instances when individuals meet all entry criteria except one that relates to transient clinical circumstances (eg, body temperature elevation or recent use of excluded medication[s] or vaccine[s]). Under these circumstances, eligibility for trial enrollment may be considered if the appropriate window for delay has passed, inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

[1] Defined as status post onset of menarche and not meeting any of the following conditions: menopausal for at least 2 years, status after bilateral tubal ligation for at least 1 year, status after bilateral oophorectomy for at least 1 year or status after hysterectomy.

[2] One or more of the following: hormonal contraceptives (such as oral, injection, transdermal patch, implant, cervical ring), barrier method (condom with spermicide or diaphragm with spermicide) every time during intercourse, intrauterine device, monogamous relationship with vasectomized partner (partner must have been vasectomized for at least 6 months prior to the subject's enrollment [Day 1 (M0)]). Other contraceptive methods may be considered in agreement with the sponsor and will be approved by the appropriate ethics committee.

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Criteria for delay of vaccination:

After enrollment, subjects may encounter clinical circumstances that warrant a delay in the administration of trial vaccine. These situations are listed below. In the event that a subject meets a criterion for delay of trial vaccine administration, the subject may receive the trial vaccine once the window for delay has passed as long as the subject is otherwise eligible for trial participation.

If any of the conditions below occur at the time scheduled for the second TDV administration (Day 90 [M3]), the second dose may be administered at a later date as long as the subject is otherwise eligible to continue trial participation. In certain situations, the period of delay may lead to deviation from the time window for the second dose at Day 90 (M3). The decision to vaccinate in those situations will be made by the investigator.

The following clinical circumstances warrant a delay for administration of the vaccination (consider whether applicable as criterion for delay of trial vaccine administration or an exclusion criterion, see above):

- Subjects with a clinically active significant infection (as assessed by the investigator) or body temperature ≥38°C (100.4°F) within 3 days of the intended date of vaccination.
- Subjects who have received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in this trial or who are planning to receive any vaccine within 28 days of trial vaccine administration.
- Known or suspected altered or impaired immune function as specified under the exclusion criteria.
- Subjects who have used antipyretics and/or analgesic medications within 24 hours prior to vaccination. The reason for their use (prophylaxis versus treatment) must be documented. Trial vaccine administration should be delayed to allow for a full 24 hours to have passed between having used antipyretics and/or analgesic medications and trial vaccine administration.

Criteria for contraindication to vaccination at Day 90 (M3):

There are also circumstances under which receipt of the second trial vaccine dose at Day 90 (M3) is contraindicated in this trial. These circumstances include but are not limited to anaphylaxis or severe hypersensitivity reactions following the first TDV vaccination at Day 1 (M0). If these reactions occur, the subject must not receive the TDV vaccination at Day 90 (M3) but will be encouraged to continue trial participation for safety follow-up.

Trial Vaccine:

The investigational vaccine is Takeda's TDV, a tetravalent dengue vaccine comprised of 1 molecularly characterized, attenuated dengue virus strain, and 3 chimeric dengue virus strains with potencies of not less than 3.3, 2.7, 4.0, and 4.5 $\log_{10}$ plaque forming units per dose of TDV-1, TDV-2, TDV-3, and TDV-4, respectively.

Route of administration: SC route

Duration of the Trial and Subject Participation:

The trial duration for each subject will be at least approximately 360 days (12 months) including vaccination (Day 1 [M0] and Day 90 [M3]) and follow-up through Day 360 (M12). This duration will be approximately 14.5 months if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial (ie, up to 70 days [10 weeks] prior to Day 1 [M0]).

Criteria for Evaluation and Analyses:

Primary Endpoint:

- GMT of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).

Secondary Endpoints:

- Frequency (percentage of subjects) and magnitude (number of Spot Forming Cells [SFC]/10^6 Peripheral Blood Mononuclear Cells [PBMC]) of IFN-γ ELISpot responses to TDV using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1) and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12). Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared with background (no peptide) and ≥50 spots per 10^6 PBMC.

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- Phenotype characteristics of cellular immune responses to TDV by ICS using blood samples collected post first vaccination on Days 15, 30 (M1) and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12). Markers will include Cluster of Differentiation (CD)4, CD8, IFN-γ, tumor necrosis factor-alpha and interleukin-2. This endpoint will be evaluated in a subset of subjects with IFN- γ ELISpot responses >500 SFC/10^6 cells and availability of sufficient cells.
- Incidence, duration, and level of vaccine viremia for each of the 4 dengue serotypes measured by qRT- PCR using blood samples collected from all subjects post first vaccination on Days 6, 9, 12, 15, and 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 96, 99, 102, 105, and 120 (M4).
- Frequency and percentage of subjects with solicited local (injection site) reactions for 7 days (day of administration + 6 days) and solicited systemic events for 14 days (day of administration + 13 days) following administration of each trial vaccine dose (Day 1 [M0] and Day 90 [M3]).
- Frequency and percentage of subjects with any unsolicited AEs for 28 days (day of administration + 27 days) following administration of each trial vaccine dose (Day 1 [M0] and Day 90 [M3]).
- Frequency and percentage of subjects with SAEs throughout the trial.
- Frequency and percentage of subjects with MAAEs throughout the trial.

Exploratory Endpoints:

- Average Effective Concentration 50 ([EC_{50}], dengue RVP) of neutralizing antibodies for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Seropositivity rates (percentage of subjects) from dengue RVP for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Seropositivity rates (percentage of subjects) from dengue RVP for multiple (2, 3 or 4) dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Average number of memory B cells expressing type-specific and cross-reactive dengue-specific antibodies/10^6 PBMC measured by Quad-color FluoroSpot using blood samples collected from all subjects post first vaccination on Day 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 180 (M6), 270 (M9), and 360 (M12).
- Average concentration (relative units/mL) of anti-dengue NS1 antibodies for each of the 4 dengue serotypes measured by ELISA using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Epitope mapping of the IFN-γ ELISpot responses to TDV on an individual level using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12). This endpoint will be evaluated in a subset of subjects with IFN- γ ELISpot responses >500 SFC/10^6 cells and availability of sufficient cells.
- Gene expression profiles on an individual level using blood samples collected from all subjects post first vaccination on Days 4 and 6; pre second vaccination (Day 90 [M3]) and on Days 94 and 96.

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Statistical Considerations:

Summaries will be provided by trial group (Group 1 and Group 2) and further for Group 2 by subgroups (DENV- 1 or DENV-3 seropositive). Unless specified otherwise, number of subjects with non-missing observations, mean, SD, median, minimum and maximum will be presented for continuous data. Frequency and percent will be presented for categorical data.

Analysis Sets

Safety Set: The Safety Set will consist of all subjects who received at least 1 dose of trial vaccine.

Full Analysis Set (FAS): The FAS will include all subjects who received at least 1 dose of trial vaccine and for whom a valid pre-dose and at least one valid post-dose blood sample is taken.

Per–Protocol Set (PPS): The PPS will include all subjects in the FAS who have no major protocol violations. The major protocol violation criteria will be defined as part of the data review prior to database lock. The categories of major protocol violations include: (1) not meeting selected entry criteria, (2) receiving prohibited therapies, (3) not receiving 2 doses of TDV, or receiving the second vaccination inadmissibly outside of the visit window, and (4) other major protocol violations that may be identified during data review prior to database lock.

Analysis of Demographics and Other Baseline Characteristics

Age, gender, race, and other Baseline characteristics will be summarized descriptively.

Immunogenicity Analysis

For the primary immunogenicity endpoint, descriptive statistics and 95% CIs will be provided for each applicable visit. Summaries will be provided by trial group (Group 1 and Group 2) and further for Group 2 by subgroups (DENV-1 or DENV-3 seropositive). The primary immunogenicity analyses will be based on the PPS; supportive analyses may be provided based on the FAS.

Similar descriptive analyses as for the primary immunogenicity endpoint will be provided for the secondary and exploratory endpoints for each applicable assay at all relevant time points, based on the PPS. Supportive analyses based on the FAS may also be provided for selected endpoints.

Antibody titers will be analyzed under log-normal distribution assumption. GMT will be calculated for each relevant time point as anti-logarithm of arithmetic mean of natural log transformed titers. The 95% CI for GMT will be calculated as the anti-log transformation of upper and lower limits for a 2-sided CI of the mean of the log-transformed titers (based on Student's t-distribution).

Other quantitative data will be summarized descriptively on the original scale.

Seropositivity rates will be accompanied with 95% CIs calculated by exact (Clopper-Pearson) method.

Relationship between immunogenicity measures will be assessed using non-parametric correlation coefficient, as appropriate. Graphical methods will also be explored. Further details will be provided in the Statistical Analysis Plan (SAP).

Handling of missing data will be described in the SAP.

Safety Analysis

All safety data will be summarized descriptively using the Safety Set, by trial group (Group 1 and Group 2) and further for Group 2 by subgroups (DENV-1 or DENV-3 seropositive).

Solicited AEs

For each solicited AE, the number and percentage of subjects with local (injection site) reactions and systemic events will be summarized by event severity for each day after each vaccination (ie, Day 1 [M0] through Day 7 for local [injection site] reactions and Day 1 [M0] through Day 14 for systemic events), and overall. Summaries of first onset of each event and the number of days subjects reported experiencing each event will also be provided. For subjects with more than 1 episode of the same event, the maximum severity will be used for tabulations.

Persistent/prolonged solicited local (injection site) reactions or systemic events continuing on Day 8 or Day 15, respectively, following each trial vaccination will be assessed separately. Unless otherwise specified, these events will not be included in the analyses/tabulations of unsolicited AEs and will have separate listings.

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Unsolicited AEs

Unsolicited AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), and summarized by System Organ Class (SOC) and Preferred Term (PT). Unsolicited AEs will be summarized for 28 days following each vaccination (day of vaccination + 27 days) as follows: by PT including events with frequency greater than a pre-defined threshold (the percentage will be specified in the SAP); by SOC and PT; by SOC, PT, and severity; and by SOC, PT, and relationship to TDV. Subjects reporting more than 1 occurrence for the term (level) being summarized will be counted only once.

AEs leading to trial vaccine withdrawal or trial discontinuation will be collected and summarized for the entire trial period.

SAEs and MAAEs

SAEs and MAAEs will be coded using MedDRA, and summarized by SOC and PT for the entire trial period.

Other Safety Observations

Vaccine viremia (incidence, duration, and level) will be evaluated descriptively at all applicable time points.

Relationship between vaccine viremia and immunogenicity measures will be assessed using non-parametric correlation coefficient, as appropriate. Graphical methods will also be explored. Further details will be provided in the SAP.

Sample Size Justification:

This trial is designed for a descriptive evaluation of the endpoints that does not require hypotheses testing. Therefore, the sample size was not determined based on formal statistical power calculations.

Interim Analysis:

No interim analysis is planned.

Data Monitoring Committee (DMC):

A DMC will have oversight of this trial. The DMC functions at a program level and further information is available in the DMC Charter.

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2.1 Schedule of Trial Procedures

Table 2.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 [M12])

	Screening visit [a]	V1	V2	V3	V4	V5	V6	V7	V8	V9
		D1 M0	D4	D6	D9	D12	D15	D30 M1	D60 M2	D90 M3
Visit window (days)	**Up to 70 days (10 weeks) prior to D1 (M0)**	**1 day (±NA)**	**4 days (±1) after V1**	**6 days (±1) after V1**	**9 days (±1) after V1**	**12 days (±1) after V1**	**15 days (-1/+2) after V1**	**30 days (-1/+7) after V1**	**60 days (±5) after V1**	**90 days (-4/+7) after V1**
Informed consent	X	X [b]								
Assessment of eligibility criteria [c]	X	X								
Trial group assignment (flavivirus-naïve or dengue-immune) [d]		X								
Demographics	X	X [e]								
Medical history	X	X								
Prior medication/vaccination	X	X								
Concomitant medications/vaccinations [f]	X	X	X	X	X	X	X	X	X	X
Review of systems		X								X
Complete physical examination [g]	X	X								X
Targeted physical examination [h]								X		
Vital signs [i]		X	X	X	X	X	X	X	X	X
Pregnancy test [j]	X	X								X
Pregnancy avoidance guidance [k]		X	X	X	X	X	X	X	X	X
Blood sampling for serological testing (2.5 mL)	X									
Blood sample for humoral immune response (20 mL) [l, m]		X					X	X	X	X

Footnotes are on last table page.

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Table 2.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 [M12]) (continued)

		Screening visit [a]	V1	V2	V3	V4	V5	V6	V7	V8	V9
			D1 M0	D4	D6	D9	D12	D15	D30 M1	D60 M2	D90 M3
Visit window (days)		Up to 70 days (10 weeks) prior to D1 (M0)	1 day (±NA)	4 days (±1) after V1	6 days (±1) after V1	9 days (±1) after V1	12 days (±1) after V1	15 days (-1/+2) after V1	30 days (-1/+7) after V1	60 days (±5) after V1	90 days (-4/+7) after V1
Blood sample for T cell-mediated immune response (60 mL) [l]			X					X	X	X	X
Blood sample for B cell-mediated immune response (30 mL) [l]			X						X		X
Blood sample for innate immune response (5 mL) [l]			X	X	X						X
Blood sample for vaccine viremia (5 mL) [l]			X		X	X	X	X	X		X
Check criteria for delay of trial vaccine administration			X								X
Check contraindications for trial vaccine administration			X								X
Trial vaccine administration [n]			X								X
Injection site evaluation [o]			X								X
Diary card [p]	Distribution		X								X
	Review/collection of solicited and unsolicited AEs			X	X	X	X	X	X		
AEs leading to trial vaccine withdrawal or trial discontinuation, SAEs, MAAEs [q]			X	X	X	X	X	X	X	X	X

Footnotes are on last table page.

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Table 2.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 [M12]) (continued)

	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19
	D94	D96	D99	D102	D105	D120 M4	D150 M5	D180 M6	D270 M9	D360 [r] M12
Visit window (days)	4 days (±1) after V9	6 days (±1) after V9	9 days (±1) after V9	12 days (±1) after V9	15 days (-1/+2) after V9	30 days (-1/+7) after V9	60 days (±5) after V9	90 days (±7) after V9	180 days (-7/+14) after V9	270 days (±14) after V9
Concomitant medications/vaccinations [e]	X	X	X	X	X	X	X	X	X	X
Targeted physical examination [g]						X				X
Vital signs [h]	X	X	X	X	X	X	X	X	X	X
Pregnancy avoidance guidance [j]	X	X	X	X	X	X				
Blood sample for humoral immune response (20 mL) [l]					X	X	X	X	X	X
Blood sample for T cell-mediated immune response (60 mL)					X	X	X	X	X	X
Blood sample for B cell-mediated immune response (30 mL)					X	X		X	X	X
Blood sample for innate immune response (5 mL)	X	X								
Blood sample for vaccine viremia (5 mL)		X	X	X	X	X				
Diary card [p] Review/collection of solicited and unsolicited AEs	X	X	X	X	X	X				
AEs leading to trial vaccine withdrawal or trial discontinuation, SAEs, MAAEs [q]	X	X	X	X	X	X	X	X	X	X

AEs=Adverse Events, D=Day, M=Month, MAAEs=Medically Attended Adverse Events, NA=Not Applicable, SAEs= Serious Adverse Events, V=Visit
Note: When a site visit cannot be carried out due to the COVID-19 pandemic, telephone contacts will be made for subjects who are still under monitoring for safety reporting.
(a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.
(b) Up to 28 days prior to Day 1 (Month 0 [M0]).

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(c) After informed consent has been obtained, eligibility of the subject will be assessed by review of inclusion/exclusion criteria at Screening or prior to TDV administration on Day 1 (M0), as applicable.
(d) Subjects will be enrolled in Group 1 (flavivirus-naïve subjects) or Group 2 (dengue-immune subjects with serology consistent with primary infection with either wild type dengue virus [DENV]-1 or DENV-3) based on serological testing performed either by the trial center prior to and outside the scope of this trial or within the scope of this trial (up to 70 days [10 weeks] prior to Day 1 [M0]).
(e) Not applicable if a Screening visit has been performed.
(f) All medications and vaccine history from 1 month (minimum 28 days) prior to administration of each trial vaccine dose up to 1 month (minimum 28 days) thereafter, steroids and immunostimulants within 60 days prior to Day 1 (M0), immunoglobulins and blood products within 3 months prior to Day 1 (M0), and immunosuppressive therapy within 6 months prior to Day 1 (M0). Concomitant medication/vaccination will be collected throughout the trial.
(g) Physical examination including measurement of weight and height; Body Mass Index (BMI) will be calculated. Measurement of height is only required at Screening or at Day 1 (M0), as applicable.
(h) Subjects may undergo a targeted symptom-directed physical examination. Clinically significant changes from the Baseline examination should be recorded in the subject's source documents and electronic Case Report Form (eCRF).
(i) Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature.
(j) Pregnancy testing (serum or urine) for females of childbearing potential. Results must be confirmed and documented as negative prior to each trial dose administration. Additional pregnancy tests may be performed during the trial if deemed necessary by the investigator.
(k) Females of childbearing potential who are sexually active will be reminded during trial visits to adhere to acceptable contraceptive methods up to 6 weeks after the last dose of TDV (Day 90 [Month 3] (M3]) + 6 weeks). Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process and will be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy and donation of ova. During the course of the trial, subjects of childbearing potential will receive continued guidance with respect to the avoidance of pregnancy.
(l) All blood samples on days of vaccination (Day 1 [M0] and Day 90 [M3]) should be taken prior to administration of TDV.
(m) Dengue neutralizing antibodies and anti-dengue non-structural protein 1 (NS1) antibodies for all subjects, and T cell epitope mapping in a subset of subjects with Interferon-gamma Enzyme-Linked Immunospot (IFN-γ ELISpot) responses >500 Spot Forming Cells/106 cells and availability of sufficient cells).
(n) Subjects will receive TDV by subcutaneous injection.
(o) Injection site pain, erythema, and swelling assessed by trial staff for 30 minutes post-vaccination.
(p) Diary cards (paper or electronic) will be distributed for the recording of 1) solicited AEs including solicited local (injection site) reactions for 7 days (day of administration + 6 days) and solicited systemic events for 14 days (day of administration + 13 days) following administration of each trial vaccine dose, and 2) unsolicited AE for 28 days (day of administration + 27 days) following administration of each trial vaccine dose. The investigator will categorize events by severity (mild, moderate or severe) and will assess causality to vaccine administration ("related" or "not related"). For persistent/prolonged solicited local (injection site) reactions or systemic events observed as continuing on Day 8 or 15, respectively, following each trial vaccination, the end date will be captured on the "Adverse Event" eCRF to permit a separate analysis from the unsolicited AEs. Any solicited local (injection site) reaction or systemic event that resolves before 8 or 15 days, respectively, following each trial vaccination, but recurs at a later time (ie, if discontinues), should be recorded as an unsolicited AE on the "Adverse Event" eCRF.
(q) MAAEs and SAEs will be collected for the trial duration.
(r) The Final Visit will be performed on Day 360 (Month 12 [M12]). If a subject terminates trial participation earlier, Day 360 (M12) procedures should be performed at their last trial visit, if possible.

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3.0 TRIAL REFERENCE INFORMATION

3.1 Trial-Related Responsibilities

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

3.2 Principal Investigator

Selection criteria for the Principal Investigator will include significant knowledge of the trial protocol, the investigational vaccine, their expertise in the therapeutic area and the conduct of clinical research as well as trial participation. Takeda will select a Signatory Investigator from the investigators who participate in the trial. The Signatory Investigator will be required to review and sign the clinical protocol. The Signatory Investigator will also be required to review and sign the Clinical Study Report (CSR) and by doing so agrees that it accurately describes the results of the trial.

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3.3 List of Abbreviations

AE(s)	Adverse Event(s)
BMI	Body Mass Index
CD	Cluster of Differentiation
CFR	Code of Federal Regulations
CSR	Clinical Study Report
CYD-TDV	Chimeric Yellow fever virus Dengue virus-Tetravalent Dengue Vaccine
DENV	Wild type dengue virus
DENV-1, -2, -3, -4	Wild type dengue virus serotypes 1, 2, 3, and 4
DHF	Dengue Hemorrhagic Fever
DMC	Data Monitoring Committee
DSS	Dengue Shock Syndrome
E	Envelope
EC_{50}	Effective Concentration 50
eCRF	electronic Case Report Form
ELISA	Enzyme-Linked ImmunoSorbent Assay
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMT	Geometric Mean Titers
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
ICS	Intracellular Cytokine Staining
IEC	Independent Ethics Committee
IFN-γ	Interferon-gamma
IFN-γ ELISpot	Interferon-gamma Enzyme-Linked Immunospot
Inc	Incorporated
IND	Investigational New Drug
IRB	Institutional Review Board
JE	Japanese Encephalitis
M0, 1, 2, 3, 4, 5, 6, 9, 12	Month 0, 1, 2, 3, 4, 5, 6, 9, 12
MAAEs	Medically Attended Adverse Events

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MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Agency of the United Kingdom
MNT_{50}	Microneutralization Test 50%
NS1	Non-structural protein 1
PBMC	Peripheral Blood Mononuclear Cells
PDK	Primary Dog Kidney
PMDA	Pharmaceuticals and Medical Devices Agency of Japan
PPS	Per-Protocol Set
prM	pre-Membrane
PT	Preferred Term
qRT-PCR	quantitative Reverse Transcription-Polymerase Chain Reaction
QTL	Quality Tolerance Limits
RVP	Reporter Virus Particle
SAE(s)	Serious Adverse Event(s)
SAP	Statistical Analysis Plan
SC	Subcutaneous
SFC	Spot Forming Cells
SOC	System Organ Class
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TDV	Takeda's Tetravalent Dengue Vaccine Candidate Tetravalent Dengue Vaccine Candidate
TDV-1	Dengue serotypes 2/1 chimeric strain
TDV-2	Molecularly characterized, attenuated dengue serotype 2 strain
TDV-3	Dengue serotypes 2/3 chimeric strain
TDV-4	Dengue serotypes 2/4 chimeric strain
US(A)	United States (of America)
WHO	World Health Organization
WN	West Nile
YF	Yellow Fever

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3.4 Corporate Identification

TV Takeda Vaccines, Inc.

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4.0 INTRODUCTION

4.1 Background

Dengue fever is caused by infection with the wild type dengue virus (DENV), a ribonucleic acid virus that occurs as 4 recognized serotypes, DENV-1, DENV-2, DENV-3, or DENV-4. These 4 DENVs are transmitted to humans by mosquitoes (primarily *Aedes aegypti*), and are endemic in Asia, Central and South America, the Caribbean, the Pacific Islands, and parts of Africa. There are an estimated 390 million dengue infections per year worldwide, which is close to 4 times the previous World Health Organization (WHO) estimate of 50 to 100 million cases. Every year, around 500,000 cases of Dengue Hemorrhagic Fever (DHF) require hospitalization with an estimated death rate of 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection [3-6].

Dengue fever is clinically defined as an acute febrile illness with 2 or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leukopenia, and occurrence at the same location and time as other confirmed cases of dengue fever. The most severe forms of dengue infection – DHF and Dengue Shock Syndrome (DSS) – are life threatening. Primary infection with any one of the 4 dengue serotypes is thought to result in life-long protection from re-infection by the same serotype but does not protect against a secondary infection by one of the other 3 dengue serotypes which may lead to an increased risk of severe disease (DHF/DSS) [5-8].

Treatment of dengue fever is based solely on medical management of signs and symptoms, with fluid replacement required for hemorrhagic or shock cases. An antiviral therapy for DENV infection is not available at this time. Preventive measures that rely on mosquito control and individual protection are of limited efficacy, complex to implement and questionable in terms of cost-effectiveness. There is a great unmet global public health need for a safe and effective vaccine to reduce the morbidity and mortality associated with dengue disease. Vaccine development has focused on tetravalent vaccines that provide protection against all 4 dengue serotypes simultaneously since all 4 dengue serotypes commonly co-circulate in endemic areas [3-9]. A first dengue vaccine (Chimeric Yellow fever virus Dengue virus-Tetravalent Dengue Vaccine [CYD-TDV]) has been approved (year 2015) in some countries in Asia and Latin America [10]. Initial findings showed that vaccine efficacy was different between serotypes and depended on dengue pre-exposure status [11]. Additionally, recent analyses found that people who had not been infected by dengue virus before vaccination had a higher risk of getting severe disease when they were infected with dengue virus after vaccination with CYD- TDV [12]. Hence, there is a continued unmet public health need for safer and more efficacious dengue vaccines [13].

Takeda's Tetravalent Dengue Vaccine Candidate (TDV) - Background:

Takeda's TDV consists of 1 molecularly characterized, attenuated dengue serotype 2 virus strain and 3 chimeric recombinant dengue virus strains expressing surface antigens corresponding to DENV serotypes 1–4. The dengue serotype 2 strain (TDV-2) is based upon the attenuated laboratory-derived DENV-2 virus strain, originally isolated at Mahidol University, Bangkok,

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Thailand and generated by 53 serial passages in Primary Dog Kidney (PDK) cells (DENV- 2 PDK- 53) [14]. The chimeric, attenuated vaccine strains for dengue serotypes 1, 3 and 4 were engineered by substituting the structural genes, pre-Membrane (prM) and Envelope (E), of TDV-2 with the prM and E genes of the DENV virus strains, DENV-1 16007, DENV- 3 16562 or DENV-4 1036 virus, respectively [15, 16]. Thus, TDV is comprised of 4 dengue virus strains: a molecularly characterized, attenuated dengue serotype 2 strain (TDV-2), a dengue serotypes 2/1 chimeric strain (TDV-1), a dengue serotypes 2/3 chimeric strain (TDV-3), and a dengue serotypes 2/4 chimeric strain (TDV-4).

Nonclinical studies carried out in mice and nonhuman primates have demonstrated an acceptable safety, immunogenicity, and efficacy profile of Takeda's TDV. Additionally, data from completed phase 1 and phase 2 clinical trials in humans have shown satisfactory reactogenicity, safety and immunogenicity profiles of Takeda's TDV in healthy adults in nonendemic areas as well as in healthy adults and children in endemic areas in Asia and Latin America. Ongoing and completed phase 2 clinical trials have enabled the selection of a final TDV dose (lyophilized formulation), and a 2-dose vaccination regimen (2 single doses) administered 3 months (ie, 90 days) apart by subcutaneous (SC) injection for use in the ongoing pivotal program.

The current IB contains additional product information and a more detailed review of nonclinical studies and clinical trials.

4.2 Rationale for the Proposed Trial

The purpose of this trial is to characterize the magnitude, quality, and evolution over time of humoral and cell-mediated immune responses to TDV when administered as a 2-dose vaccination regimen (2 single doses 3 months [ie, 90 days] apart). Furthermore, this trial aims to characterize the relationships between TDV-specific innate, humoral and cellular immune responses. This characterization will aid in the understanding of vaccine efficacy and in the identification of potential determinants for protection against all 4 dengue serotypes in the ongoing phase 3 TDV clinical program. This trial will be conducted at trial centers in the United States of America involving healthy adults who are either flavivirus-naïve or seropositive for DENV-1 or DENV-3 (ie, serology consistent with primary infection with either DENV-1 or DENV-3). The serological status of subjects will either have been determined by the trial center prior to and outside the scope of this trial or will be determined through screening within the scope of this trial.

Although certain parameters of TDV-specific humoral and cell-mediated immune responses have been evaluated to some extent in phase 1 and other phase 2 clinical trials with TDV, the present trial is the only one that will allow a comprehensive analysis of TDV-specific humoral, T cell-mediated, B cell-mediated, innate, and early immune responses. In particular, these responses following 2 doses of TDV in both flavivirus-naïve and dengue-immune adults have not been evaluated yet in any other single trial in the TDV clinical program. Furthermore, conduct of the present trial would create a subject population, potentially eligible for inclusion in a future DENV-1/DENV-3 human challenge trial. Such a trial may be considered at a later date by the sponsor if deemed appropriate to further guide TDV development.

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The present trial will be conducted in accordance with the protocol, ICH and GCP Guidelines, and applicable regulatory requirements [2].

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5.0 TRIAL OBJECTIVES AND ENDPOINTS

5.1 Objectives

5.1.1 Primary Objective

- To assess the neutralizing antibody response (Geometric Mean Titers [GMT]) against each dengue serotype post-vaccination (Microneutralization Test 50% [MNT_{50}]).

5.1.2 Secondary Objectives

Immunogenicity

- To assess the magnitude (Interferon-gamma Enzyme-Linked Immunospot [IFN-γ ELISpot]) and polyfunctionality (Intracellular Cytokine Staining [ICS]) of the T cell-mediated immune response post-vaccination.
- To assess vaccine viremia post-vaccination and the integrity of main attenuation mutations.

Safety

- To describe the safety of 2 doses of TDV in healthy subjects aged 18 to 60 years (inclusive).

5.1.3 Exploratory Objectives

- To assess the neutralizing antibody response against each dengue serotype post-vaccination (Reporter Virus Particle [RVP] test).
- To assess the B cell-mediated immune response post-vaccination (Quad-color FluoroSpot).
- To assess the anti-dengue non-structural protein 1 (NS1) antibody response post-vaccination (Enzyme-Linked ImmunoSorbent Assay [ELISA]).
- To map the epitopes of the dengue antibodies post-vaccination (IFN-γ ELISpot response).
- To assess the innate immune response post-vaccination.
- To characterize the relationships between TDV-specific innate, humoral and cellular immune responses on an individual level post-vaccination (MNT_{50}, dengue RVP, Quad color FluoroSpot, NS1 antibody ELISA, IFN- γ ELISpot, ICS, and exploratory assays).
- To characterize the relationships between vaccine viremia (quantitative Reverse Transcription-Polymerase Chain Reaction [qRT-PCR]) and TDV-specific innate, humoral and cellular immune responses on an individual level post-vaccination (MNT_{50}, dengue RVP, Quad color FluoroSpot, NS1 antibody ELISA, IFN-γ ELISpot, ICS, and exploratory assays).

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5.2 Endpoints

5.2.1 Primary Endpoint

- GMT of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (Month 1 [M1]), and 60 (Month 2 [M2]); pre second vaccination (Day 90 [Month 3, (M3)]) and on Days 105, 120 (Month 4 [M4]), 150 (Month 5 [M5]), 180 (Month 6 [M6]), 270 (Month 9 [M9]), and 360 (Month 12 [M12]).

5.2.2 Secondary Endpoints

- Frequency (percentage of subjects) and magnitude (number of Spot Forming Cells [SFC]/10^6 Peripheral Blood Mononuclear Cells [PBMC]) of IFN-γ ELISpot responses to TDV using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1) and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12). Cellular immune response is defined as an IFN- γ ELISpot response that is >3 times higher compared with background (no peptide) and ≥50 spots per 10^6 PBMC.
- Phenotype characteristics of cellular immune responses to TDV by ICS using blood samples collected post first vaccination on Days 15, 30 (M1) and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12). Markers will include Cluster of Differentiation (CD)4, CD8, IFN-γ, tumor necrosis factor-alpha and interleukin-2. This endpoint will be evaluated in a subset of subjects with IFN- γ ELISpot responses >500 SFC/10^6 cells and availability of sufficient cells.
- Incidence, duration, and level of vaccine viremia for each of the 4 dengue serotypes measured by qRT-PCR using blood samples collected from all subjects post first vaccination on Days 6, 9, 12, 15, and 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 96, 99, 102, 105, and 120 (M4).
- Frequency and percentage of subjects with solicited local (injection site) reactions for 7 days (day of administration + 6 days) and solicited systemic events for 14 days (day of administration + 13 days) following administration of each trial vaccine dose (Day 1 [M0] and Day 90 [M3]).
- Frequency and percentage of subjects with any unsolicited Adverse Events (AEs) for 28 days (day of administration + 27 days) following administration of each trial vaccine dose (Day 1 [M0] and Day 90 [M3]).
- Frequency and percentage of subjects with SAEs throughout the trial.
- Frequency and percentage of subjects with Medically Attended Adverse Events (MAAEs) throughout the trial.

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5.2.3 Exploratory Endpoints

- Average effective concentration 50 ([EC_{50}], dengue RVP) of neutralizing antibodies for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Seropositivity rates (percentage of subjects) from dengue RVP for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Seropositivity rates (percentage of subjects) from dengue RVP for multiple (2, 3 or 4) dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Average number of memory B cells expressing type-specific and cross-reactive dengue-specific antibodies/10^6 PBMC measured by Quad-color FluoroSpot using blood samples collected from all subjects post first vaccination on Day 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 180 (M6), 270 (M9), and 360 (M12).
- Average concentration (relative units/mL) of anti-dengue NS1 antibodies for each of the 4 dengue serotypes measured by ELISA using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Epitope mapping of the IFN-γ ELISpot responses to TDV on an individual level using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12). This endpoint will be evaluated in a subset of subjects with IFN- γ ELISpot responses >500 SFC/10^6 cells and availability of sufficient cells.
- Gene expression profiles on an individual level using blood samples collected from all subjects post first vaccination on Days 4 and 6; pre second vaccination (Day 90 [M3]) and on Days 94 and 96.

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6.0 TRIAL DESIGN AND DESCRIPTION

6.1 Trial Design

This is an open-label, phase 2 trial in 44 healthy adult subjects aged 18 to 60 years (inclusive) to investigate the immunogenicity and safety of SC administration of a 2-dose regimen of TDV.

Subjects will be enrolled in 2 trial groups based on results from serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 70 days [10 weeks] prior to Day 1[M0]):

- Group 1: approximately 22 flavivirus-naïve subjects.
- Group 2: approximately 22 dengue-immune subjects (ie, subjects with serology consistent with primary infection by DENV-1 and subjects with serology consistent with primary infection by DENV-3).

Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.

TDV will be administered on Day 1 (M0) and on Day 90 (M3) in both Groups 1 and 2.

The blood sampling schedule is summarized in Figure 6.a and Table 2.a. In compliance with the National Institutes of Health guidance, the total amount of blood that will be drawn from trial participants over an 8-week period will not exceed 10.5 mL/kg or 550 mL (whichever is smaller) [17].

All subjects will be followed-up for 9 months post second vaccination so the trial duration will be approximately 360 days (12 months) or 14.5 months for each subject depending on whether serological testing with regard to the inclusion criteria for the trial is performed outside or within the scope of this trial, respectively.

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

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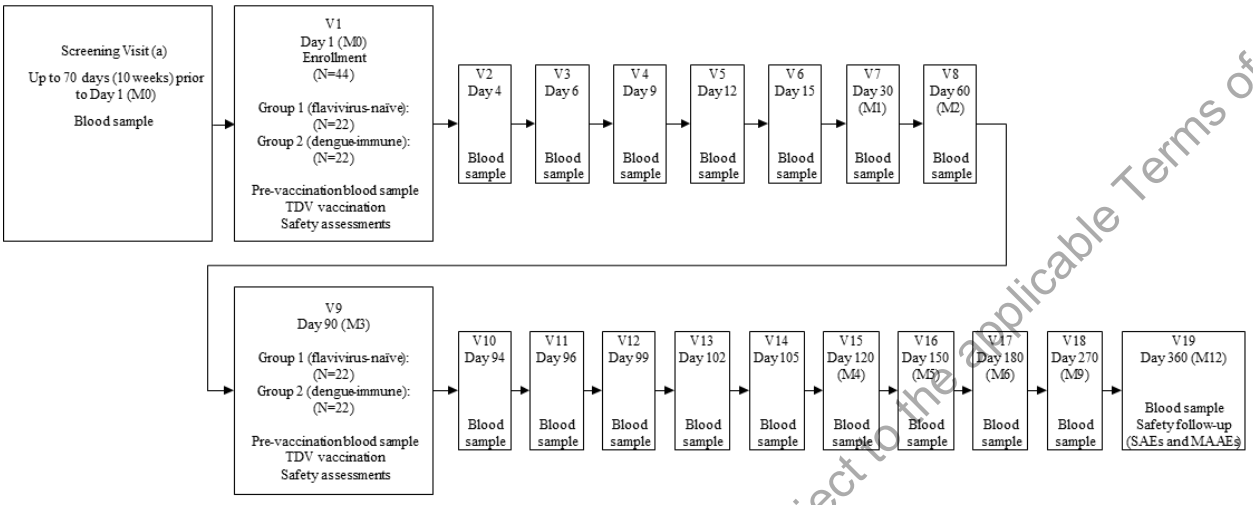
Figure 6.a Schematic of Trial Design



Note: SAEs and MAAEs are collected continuously throughout the trial.

Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.

M=month, MAAE=medically attended adverse event, SAE=serious adverse event, V=visit

(a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.

Immunogenicity evaluations (see also Section 9.1.7):

- Dengue neutralizing antibodies will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- T cell-mediated immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- B cell-mediated immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Day 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 180 (M6), 270 (M9), and 360 (M12).
- T cell epitopes will be mapped using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Anti-dengue NS1 antibodies will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).

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- The innate immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 4 and 6; pre second vaccination (Day 90 [M3]) and on Days 94 and 96.

Safety evaluations:

- Diary cards (paper or electronic) will be distributed for the recording of:
  - Solicited AEs:
    - Solicited local (injection site) reactions for 7 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 6 days). These will include: injection site pain, injection site erythema, and injection site swelling.
    - Solicited systemic events for 14 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 13 days). These will include: fever, headache, asthenia, malaise, and myalgia.
  - Unsolicited AEs for 28 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 27 days).
- SAEs, MAAEs, and AEs leading to trial vaccine withdrawal or trial discontinuation will be collected for the trial duration. MAAEs are defined as AEs leading to an unscheduled medical visit to or by a healthcare professional including visits to an emergency department, but not fulfilling seriousness criteria.

Vaccine viremia evaluation:

- Vaccine viremia will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 6, 9, 12, 15, and 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 96, 99, 102, 105, and 120 (M4).

Data collection will be by electronic Case Report Form (eCRF).

6.2 Justification for Trial Design, Dose, and Endpoints

The trial design and the collection of solicited AEs (local [injection site] reactions and systemic events), unsolicited AEs (non-serious AEs and SAEs), and MAAEs following trial dose administration are consistent with vaccine evaluation trials.

Ongoing and completed phase 2 trials have enabled the selection of a final TDV dose (lyophilized formulation) and a 2-dose vaccination regimen 3 months apart by SC injection for use in Takeda's dengue pivotal study program.

The timing of the primary and secondary endpoints after vaccination is consistent with previous trials with TDV. Dengue neutralizing antibodies have been generally accepted as the immune response endpoint for dengue vaccine trials.

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The blood sampling schedule and exploratory analyses will allow a comprehensive analysis of the kinetics (onset, longevity) of the various immune responses.

Considering that the measurements of the immune responses are unlikely to be influenced by the knowledge of the trial group assignment, a placebo group for comparison has not been included in this trial.

Involvement of healthy adults who are either flavivirus-naïve or seropositive for DENV-1 or DENV-3 in the present trial could create a subject population eligible for participation in a potential future DENV-1/DENV-3 human challenge trial if the sponsor deems this necessary in the further development of the trial vaccine.

Justification of the sample size is included in Section 13.3. The rationale for the proposed trial is given in Section 4.2.

The current IB contains additional product information and a more detailed review of nonclinical studies and clinical trials.

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

The trial duration for each subject will be at least approximately 360 days (12 months) including vaccination (Day 1 [M0] and Day 90 [M3]) and follow-up through Day 360 (M12). This duration will be approximately 14.5 months if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial (ie, up to 70 days [10 weeks] prior to Day 1 [M0]).

6.4 Premature Termination or Suspension of Trial or Investigational Site

6.4.1 Criteria for Premature Termination or Suspension of the Trial

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

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

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6.4.2 Criteria for Premature Termination or Suspension of Investigational Sites

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

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

In the event that the sponsor, an Institutional Review Board (IRB)/Independent Ethics Committee (IEC) or regulatory authority elects to terminate or suspend the trial or the participation of the investigational site, a trial-specific procedure for early termination or suspension will be provided by the sponsor; the procedure will be followed by the investigational site during the course of termination or trial suspension.

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7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF SUBJECTS

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

7.1 Inclusion Criteria

Subject eligibility is determined according to the following criteria:

1. The subject is aged ≥18 to ≤60 years.
2. Male or female.
3. Subjects who are in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs) and clinical judgment of the investigator.
4. Group 1 only: immunologically naïve to dengue, Zika, Yellow Fever (YF), Japanese Encephalitis (JE), West Nile (WN) (based on negative results for detection of anti-DENV, anti-Zika, anti-YF, anti-JE, anti-WN antibodies) as documented by serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 70 days [10 weeks] prior to Day 1 [M0]).
5. Group 2 only: serology consistent with primary infection with either DENV-1 or DENV-3 (defined as detectable neutralizing antibodies against DENV-1 or DENV-3 only, or titers for DENV-1 or DENV-3 ≥4-times higher than titers for the 2 other dengue serotypes) as documented by serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 70 days [10 weeks] prior to Day 1 [M0]).
6. The subject signs and dates a written, informed consent and any required privacy authorization prior to the initiation of any trial procedures, after the nature of the trial has been explained according to local regulatory requirements.
7. Subjects who can comply with trial procedures and are available for the duration of follow-up.

7.2 Exclusion Criteria

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

1. Subjects with a clinically active significant infection (as assessed by the investigator) or body temperature ≥38°C (100.4°F) within 3 days of the intended date of vaccination (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration, see Section 7.3).
2. Subjects with a known hypersensitivity or allergy to any of the trial vaccine components (including excipients).
3. Subjects with behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.

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4. Subjects with any history of progressive or severe neurologic disorder, seizure disorder or neuro-inflammatory disease (eg, Guillain-Barré syndrome).
5. Subjects with any illness, or history of any illness that, in the opinion of the investigator, might interfere with the results of the trial or pose additional risk to the subjects due to participation in the trial.
6. Known or suspected impairment/alteration of immune function (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration, see Section 7.3), including:
   a) Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks and/or ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0) (use of inhaled, intranasal, or topical corticosteroids is allowed).
   b) Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks and/or ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0).
   c) Administration of immunoglobulins and/or any blood products within 3 months prior to Day 1 (M0) or planned administration during the trial.
   d) Receipt of immunostimulants within 60 days prior to Day 1 (M0).
   e) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (M0).
   f) Known Human Immunodeficiency Virus (HIV) infection or HIV-related disease.
   g) Hepatitis C virus infection.
   h) Genetic immunodeficiency.
7. Abnormalities of splenic or thymic function.
8. Subjects with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
9. Subjects with any serious chronic or progressive disease according to the judgment of the investigator (eg, neoplasm, hematologic malignancies, insulin dependent diabetes; cardiac, renal, or hepatic disease).
10. Subjects with Body Mass Index (BMI) greater than or equal to 35 kg/m^2 (=weight in kg/ height in $meters^2$).
11. Subjects participating in any clinical trial with another investigational product 30 days prior to Day 1 (M0) or intending to participate in another clinical trial at any time during the conduct of this trial.
12. Subjects who have received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in this trial or who are planning to receive any

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vaccine within 28 days of trial vaccine administration (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration, see Section 7.3).

13. Subjects who have used antipyretics and/or analgesic medications within 24 hours prior to vaccination. The reason for their use (prophylaxis versus treatment) must be documented. Trial entry should be delayed to allow for a full 24 hours to have passed since last use of antipyretics and/or analgesic medications (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration, see Section 7.3).
14. Subjects involved in the trial conduct or their first-degree relatives.
15. Subjects with history of substance or alcohol abuse within the past 2 years.
16. Female subjects who are pregnant or breastfeeding.
17. Female subjects of childbearing potential who are sexually active with men and have not used any of the "acceptable contraceptive methods" for at least 2 months prior to Day 1 (M0).
    a) Of "childbearing potential" is defined as status post onset of menarche and not meeting any of the following conditions: menopausal for at least 2 years, status after bilateral tubal ligation for at least 1 year, status after bilateral oophorectomy for at least 1 year or status after hysterectomy.
    b) "Acceptable birth control methods" are defined as one or more of the following:
        I. Hormonal contraceptives (such as oral, injection, transdermal patch, implant, cervical ring).
        II. Barrier method (condom with spermicide or diaphragm with spermicide) every time during intercourse.
        III. Intrauterine device.
        IV. Monogamous relationship with vasectomized partner (partner must have been vasectomized for at least 6 months prior to the subject's enrollment [Day 1 (M0)]).

    Other contraceptive methods may be considered in agreement with the sponsor and will be approved by the appropriate ethics committee.
18. Female subjects of childbearing potential who are sexually active with men and refuse to use an acceptable contraceptive method up to 6 weeks after the last dose of trial vaccine (Day 90 [M3] + 6 weeks). In addition, female subjects must be advised not to donate ova during this period (see Section 9.1.11).
19. Any positive or indeterminate pregnancy test (see Section 9.1.12).
20. Previous participation in any clinical trial of a dengue candidate vaccine, except for subjects who received placebo in those trials, or previous and planned vaccination (during the trial conduct) against dengue.

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21. Planned vaccination (during the trial conduct) against any non-dengue flavivirus (eg, Zika, YF, JE, WN, tick-borne encephalitis, or Murray-Valley encephalitis).
22. Planned travel (during the trial conduct) to any area endemic for dengue.

There may be instances when individuals meet all entry criteria except one that relates to transient clinical circumstances (eg, body temperature elevation or recent use of excluded medication[s] or vaccine[s]). Under these circumstances, eligibility for trial enrollment may be considered if the appropriate window for delay has passed, inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

7.3 Criteria for Delay of Trial Vaccine Administration

After enrollment, subjects may encounter clinical circumstances that warrant a delay in the administration of trial vaccine. These situations are listed below. In the event that a subject meets a criterion for delay of trial vaccine administration, the subject may receive the trial vaccine once the window for delay has passed as long as the subject is otherwise eligible for trial participation.

If any of the conditions below occur at the time scheduled for the second TDV administration (Day 90 [M3]), the second dose may be administered at a later date as long as the subject is otherwise eligible to continue trial participation. In certain situations, the period of delay may lead to deviation from the time window for the second dose at Day 90 (M3). The decision to vaccinate in those situations will be made by the investigator.

The following clinical circumstances warrant a delay for administration of the vaccination (consider whether applicable as criterion for delay of trial vaccine administration or an exclusion criterion, see Section 7.2):

- Subjects with a clinically active significant infection (as assessed by the investigator) or body temperature ≥38°C (100.4°F) within 3 days of the intended date of vaccination.
- Subjects who have received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in this trial or who are planning to receive any vaccine within 28 days of trial vaccine administration.
- Known or suspected altered or impaired immune function as specified under the exclusion criteria (see Section 7.2, exclusion criterion no. 6).
- Subjects who have used antipyretics and/or analgesic medications within 24 hours prior to vaccination. The reason for their use (prophylaxis versus treatment) must be documented. Trial vaccine administration should be delayed to allow for a full 24 hours to have passed between having used antipyretics and/or analgesic medications and trial vaccine administration.

7.4 Early Termination of a Subject's Trial Participation

Under some circumstances, a subject's trial participation may be terminated early. This means that no further trial procedures (including data collection) will be performed on that subject

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beyond the specific date of early termination of trial participation. The primary reason for early termination of the subject's trial participation should be documented using the following categories. While the subject has no obligation to provide a reason for withdrawing consent, attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be documented.

For screen failure subjects, refer to Section 9.1.13.

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

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

7.5 Criteria for Premature Discontinuation of Trial Vaccine Administration

Criteria for contraindication to vaccination at Day 90 (M3):

There are also circumstances under which receipt of the second trial vaccine dose at Day 90 (M3) is contraindicated in this trial. These circumstances include but are not limited to anaphylaxis or severe hypersensitivity reactions following the first TDV vaccination at Day 1 (M0). If these reactions occur, the subject must not receive the TDV vaccination at Day 90 (M3) but will be encouraged to continue trial participation for safety follow-up.

Early termination of a subject's trial participation will by default prevent the subject from receiving further doses of trial vaccine, as the subject will no longer be participating in the trial. In addition to criteria for early termination of a subject's participation (see Section 7.4), other

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situations may apply in which subjects may continue participating in the trial (eg, contributing safety data according to protocol) but trial vaccine administration is discontinued. Even if the subject is deemed ineligible to receive further doses of trial vaccine, all efforts should be made to continue the collection of safety data according to protocol.

In addition, the primary reason for premature discontinuation of trial vaccine administration should be recorded in the "End of Trial Vaccine Administration" eCRF using the following categories:

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

   If the clinical trial is prematurely terminated by the sponsor, the investigator is to promptly inform the trial subjects and local IEC/IRB and should assure appropriate follow-up for the subjects. The primary reason for early termination in this case will be "trial termination".
5. Subject´s death during trial participation.
6. Protocol deviation: A protocol deviation is any change, divergence, or departure from the trial design or procedures of a trial protocol. The subject may remain in the trial unless continuation in the trial jeopardizes the subject's health, safety or rights (see Section 7.4).
7. Pregnancy: Any subject who, despite the requirement for adequate contraception, becomes pregnant during the trial will not receive further trial vaccine administrations. Pregnant subjects should, however, be asked to continue participating in the trial contributing data to the safety follow-up according to protocol. In addition, the site should maintain contact with the pregnant subject and complete a "Clinical Trial Pregnancy Form" as soon as possible. The subject should be followed-up until the birth of the child, or spontaneous or voluntary termination; when pregnancy outcome information becomes available, the information should be captured using the same form. Data obtained from the "Clinical Trial Pregnancy Form" will be captured in the safety database.
8. Other.

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8.0 CLINICAL TRIAL MATERIAL MANAGEMENT

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

8.1 Trial Vaccine and Materials

The investigational vaccine is Takeda's TDV, a tetravalent dengue vaccine comprised of 1 molecularly characterized, attenuated dengue virus strain, and 3 chimeric dengue virus strains with potencies of not less than 3.3, 2.7, 4.0, and 4.5 $\log_{10}$ plaque forming units per dose of TDV-1, TDV-2, TDV-3, and TDV-4, respectively.

8.1.1 Dosage Form, Manufacturing, Packaging, and Labeling

Manufacturing of monovalent bulk vaccine substances of TDV, mixing of the 4 TDV vaccine substances, filling into vials, and lyophilization of TDV is done at IDT Biologika GmbH, Germany.

Lyophilized TDV is presented in a single-dose 2 mL glass vial with a grey bromo butyl rubber stopper and flip-top aluminum over seal.

TDV diluent (37 mM sodium chloride solution) is a clear, colorless solution provided in a single-use 2 mL glass vial or a single-use 1 mL glass syringe and is used to reconstitute the lyophilized TDV to deliver a 0.5 mL dose.

TDV and TDV diluent vials are packaged together into single dose dispensing cartons. The units will be labeled to contain pertinent trial information in local languages.

The sponsor will supply the trial site with TDV and TDV diluent packaged into single dose dispensing cartons. The units and cartons will be labeled with pertinent trial information in local languages. Further details can be found in the Pharmacy Manual.

8.1.2 Storage

The trial vaccine (TDV) and TDV diluent will be shipped in refrigerated containers at 2°C to 8°C. From receipt and prior to use, TDV and TDV diluent must be protected from light and stored at 2°C to 8°C in a refrigerator. Do not freeze.

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

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8.1.3 Dose and Regimen

All subjects in Groups 1 and 2 are to receive 1 trial vaccine dose on Day 1 (M0) and 1 trial vaccine dose on Day 90 [M3]).

The 0.5 mL trial vaccine doses will be prepared and administered by the pharmacist or vaccine administrator according to the instructions in the Pharmacy Manual or per sponsor instructions.

TDV will be administered by the SC route.

8.2 Trial Vaccine Assignment and Dispensing Procedures

Refer to Section 8.6 for accountability of sponsor-supplied vaccines.

If sponsor-supplied vaccine is lost or damaged, the site can request a replacement. Expired vaccines must not be administered.

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

Eligibility for subsequent trial vaccination is determined by following the criteria outlined in Section 7.3.

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

8.2.1 Precautions to be Observed when Administering the Trial Vaccine

Prior to trial vaccine administration, a subject must be determined to be eligible to receive trial vaccine and it must be clinically appropriate in the judgment of the investigator to administer the trial vaccine.

First, trial eligibility is evaluated according to the entry criteria outlined in this protocol (Sections 7.1 and 7.2). Once eligibility is confirmed, the subject is able to receive the first trial vaccination.

Prior to subsequent trial vaccine administration, site staff must determine if the subject is eligible to receive vaccination by evaluating the criteria outlined in Sections 7.3, 7.4, and 7.5.

Standard immunization practices are to be observed and care should be taken to administer the injection by the SC route. In addition, WHO recommendations to reduce anxiety and pain at the time of vaccination should be followed [18]. Before administering the trial vaccine, the vaccination site is to be disinfected with a skin disinfectant (eg, 70% alcohol). Allow the skin to dry. DO NOT inject intravascularly.

As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccination. For example,

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epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available. These rescue medications will not be supplied by the sponsor.

8.3 Randomization Code Creation and Storage

Not applicable.

8.4 Trial Vaccine Blind Maintenance

Not applicable.

8.5 Unblinding Procedure

Not applicable.

8.6 Accountability and Destruction of Sponsor-Supplied Trial Vaccine and Other Clinical Trial Materials

Vaccine supplies will be counted and reconciled at the site before being locally destroyed or returned to the sponsor or designee as noted below. The site will maintain source documents.

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

Upon receipt of sponsor-supplied trial vaccine, the investigator or designee must verify the contents of the shipments against the packing list. The verifier should ensure that the quantity is correct, the trial vaccine is received within the labeled storage conditions (ie, no cold chain break has occurred during transit), and is in good condition. If quantity and conditions are acceptable, investigator or designee will acknowledge receipt of the shipment by signing bottom half of the packing list and forward the list per instructions provided on the form.

If there are any discrepancies between the packing list versus the actual product received, the sponsor or designee must be contacted to resolve the issue. The packing list should be filed in the Pharmacy Investigator Site File by a qualified investigator designee.

The pharmacist (or designated individual) at the site must maintain 100% accountability for sponsor-supplied trial vaccine (TDV including TDV diluent) and other clinical trial material received and administered during their entire participation in the trial. Accountability includes, but is not limited to:

- Verifying that actual inventory matches documented inventory.
- Verifying that the log is completed for the vaccine lot used to prepare each dose.
- Verifying that all trial vaccine kits used are documented accurately on the log.

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- Verifying that required fields are completed accurately and legibly.

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

The pharmacist (or designated individual) at the site must record the current inventory of all sponsor-supplied vaccine (TDV including TDV diluent) on a sponsor-approved vaccine accountability log. The following information will be recorded at a minimum: protocol number and title, name of investigator, site identifier and number, description of sponsor-supplied trial vaccine, expiry and/or retest date, and amount. The log should include all required information as a separate entry for each subject to whom sponsor-supplied trial vaccine is administered.

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

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

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9.0 TRIAL PLAN

9.1 Trial Procedures

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

9.1.1 Informed Consent

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

Informed consent must be obtained prior to the subject entering into the trial, and before any protocol-directed procedures are performed.

A unique subject number will be assigned to each subject after informed consent is obtained. If all eligibility criteria are fulfilled, this subject number will be used throughout the trial. Subject numbers assigned to subjects who fail screening should not be reused (Section 9.1.13).

9.1.2 Demographics, Medical History and Prior Medications

Demographic information to be obtained will include age, sex, race, and ethnicity as described by the subject.

Medical history will also be collected, including but not limited to any medical history that may be relevant to subject eligibility for trial participation such as prior vaccinations, concomitant medications, and previous and ongoing illnesses and/or injuries. Relevant medical history can also include any medical history that contributes to the understanding of an AE that occurs during trial participation, if it represents an exacerbation of an underlying disease/preexisting problem.

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

Adverse medical occurrences emerging during the time between signing of the informed consent form and the first administration of trial vaccine will be recorded in the "Medical History" eCRF. If such an adverse medical occurrence is assessed as related to a trial procedure this should be recorded in the eCRF as an AE related to trial procedure.

All medications, vaccines and blood products taken or received by the subjects are to be collected as "Prior and Concomitant Medications" and recorded on the "Prior and Concomitant Medications" eCRF and in the subject's source document:

a) Medications: from 1 month (minimum 28 days) prior to administration of each trial vaccine dose up to 1 month (minimum 28 days) thereafter,

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b) Vaccines: from 1 month (minimum 28 days) prior to administration of each trial vaccine dose up to 1 month (minimum 28 days) thereafter,

c) Steroids and immunostimulants within 60 days prior to Day 1 (M0),

d) Immunoglobulins and blood products within 3 months prior to Day 1 (M0),

e) Immunosuppressive therapy within 6 months prior to Day 1 (M0).

The use of antipyretics and/or analgesic medications within 24 hours prior to vaccination must be identified and the reason for their use (prophylaxis versus treatment) must be documented in the subject's source documents and the eCRF. Trial vaccine administration should be delayed if subjects have used antipyretics and/or analgesic medications within 24 hours prior to vaccine administration (see Section 7.3).

Medications taken for prophylaxis are those intended to prevent the onset of AEs following vaccination. Medications taken for treatment are intended to reduce or eliminate the presence of symptoms that are present.

Prohibited therapies (see also Section 7.2):

a) Parenteral immunoglobulin preparation, blood products, and/or blood-derived products within 3 months prior to Day 1 (M0),

b) Immunosuppressive therapy within 6 months or systemic (eg, oral or parenteral) corticosteroid treatment within 60 days prior to Day 1 (M0) or immunostimulants within 60 days prior to Day 1 (M0),

c) Any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 (M0) and Day 90 (M3), and 28 days after each trial vaccination,

d) Any other dengue vaccines (investigational or licensed) for the entire trial period,

e) Receipt of any other clinical trial product within 30 days prior to Day 1 (M0).

These data must be written in the subject's source documents.

9.1.3 Documentation of Trial Entrance/Randomization

Only subjects who have a signed informed consent form, meet all of the inclusion criteria and none of the exclusion criteria are eligible for entrance into the active phase.

If the subject is found to be ineligible for entrance into the active phase, the investigator should record the primary reason for failure on the subject's screening and enrollment log.

Randomization is not applicable for the trial.

9.1.4 Physical Examination

Physical examinations must be performed by a qualified health professional in accordance with local regulations and as listed within the Site Responsibility Delegation Log. A complete physical examination will be performed at Screening (if applicable) and prior to vaccination on

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Day 1 (M0) and Day 90 (M3). A complete physical examination includes but is not limited to: auscultation of heart and lungs, palpation of the abdomen, inspection of extremities (including skin over intended vaccination site), a check of general appearance and the measurement of weight and height; BMI will be calculated. Measurement of height is only required at Screening or Day 1 (M0), as applicable. Additional physical examinations may be performed if indicated by review of the subject's medical history. The findings should be documented in the subject's source document.

Targeted symptom-directed physical examination including but not limited to measurement of vital signs (see Section 9.1.5) may be performed on Day 30 (M1), Day 120 (M4) and Day 360 (M12). Clinically significant changes from the Baseline examination should be recorded in the subject's source documents and the eCRF.

9.1.5 Vital Signs

During the physical examination, a subject should have their vital signs measured. These will include (but are not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature at all trial visits subsequent to Visit 1 (Day 1 [M0]).

9.1.6 Screening

A blood sample (2.5 mL) will be collected at the screening visit (up to 70 days [10 weeks] prior to Day 1 [M0)]). A screening visit is only applicable if serological testing with regard to the inclusion criteria (see Section 7.1) is performed within the scope of this trial.

All blood samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guideline provided to the trial site.

9.1.7 Immunogenicity Assessments

- Humoral immune responses (dengue neutralizing antibodies and anti-dengue NS1 antibodies for all subjects, and T cell epitope mapping in a subset of subjects with IFN- γ ELISpot responses >500 SFC/10^6 cells and availability of sufficient cells) will be measured using blood samples (20 mL) collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- T cell-mediated immune response (60 mL) will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- B cell-mediated immune response will be measured using blood samples (30 mL) collected from all subjects pre first vaccination (Day 1 [M0]) and on Day 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 180 (M6), 270 (M9), and 360 (M12).

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- The innate immune response will be measured using blood samples (5 mL) collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 4 and 6; pre second vaccination (Day 90 [M3]) and on Days 94 and 96.

All blood samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guideline provided to the trial site.

9.1.8 Processing, Labeling and Storage of Biological Samples

PBMC will be collected, processed, labeled and stored according to trial site Standard Operating Procedures (SOP). Refer to the SOP for detailed instructions.

All biological samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guideline provided to the site.

9.1.9 Safety Assessments

Safety assessments will include collection and recording of solicited local (injection site) reactions and systemic events, unsolicited AEs (serious and non-serious), and MAAEs. Refer to Section 10.1 for safety definitions. Details on collection and reporting of AEs are in Section 10.4.

9.1.10 Clinical Safety Laboratory Variables

Vaccine viremia will be measured using blood samples (5 mL) collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 6, 9, 12, 15, and 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 96, 99, 102, 105, and 120 (M4).

All blood samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guideline provided to the trial site.

9.1.11 Contraception and Pregnancy Avoidance Procedure

For female subjects of childbearing potential, serum or urine pregnancy testing will be performed at Screening (if applicable) and prior to each trial dose administration (Day 1 [M0] and Day 90 [M3]). Results must be confirmed and documented as negative prior to each trial dose administration. Additional pregnancy tests may be performed during the trial if deemed necessary by the investigator. Females of childbearing potential who are sexually active will be reminded during trial visits to adhere to acceptable contraceptive methods up to 6 weeks after the last dose of TDV (Day 90 [M3] + 6 weeks). Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process and will be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy and donation of ova. During the course of the trial, subjects of childbearing potential will receive continued guidance with respect to the avoidance of pregnancy as part of the trial procedures (see Section 2.1). Refer also to Section 7.2.

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9.1.12 Pregnancy

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

Any pregnancy occurring following trial vaccine administration should be reported immediately, using a "Clinical Trial Pregnancy Form", to the contact listed in the Investigator Site File.

9.1.13 Documentation of Subjects Who are Not Randomized

Investigators must account for all subjects who sign an informed consent. If the subject is found to be not eligible at Day 1 (M0), the investigator should complete the eCRF. Randomization is not applicable for the trial.

The primary reason for not receiving the trial vaccine is to be recorded in the eCRF using the following categories:

- Adverse medical occurrence prior to receipt of investigational vaccine.
- Screen failure (did not meet one or more inclusion criteria or did meet one or more exclusion criteria).
- Withdrawal by subject.
- Site terminated by sponsor.
- Trial terminated by sponsor.
- Other (specify reason).

Subject numbers assigned to enrolled subjects who are not vaccinated should not be reused.

9.2 Monitoring Subject Compliance

The investigator must record all injections of trial vaccine given to the subject in the subject's source document and the eCRF.

9.3 Schedule of Observations and Procedures

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

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9.3.1 Procedures at the Screening Visit (up to 70 days [10 weeks] prior to Day 1 [M0])

Note: A screening visit is only applicable if serological testing with regard to the inclusion criteria (see Section 7.1) is performed within the scope of this trial.

1. Before performing any trial procedure, the signed informed consent form needs to be obtained. Refer to Section 9.1.1.
2. Check inclusion and exclusion criteria. Refer to Section 7.1 and Section 7.2, respectively.
3. Collect demographic data, medical history, and prior medication/vaccination. Refer to Section 9.1.2.
4. Collect concomitant medications/vaccinations. Refer to Section 9.1.2.
5. Perform a complete physical examination. Refer to Section 9.1.4.
6. Perform pregnancy testing (serum or urine) for females of childbearing potential. Refer to Section 9.1.11.
7. Collect blood sample. Refer to Section 9.1.6.

   Blood should be taken from the subject using an aseptic venipuncture technique.

The site should schedule the next trial visit with the subject.

The subject will receive a written reminder of the next trial visit.

9.3.2 Pre-Vaccination Procedures (Day 1 [M0] and Day 90 [M3])

1. Before performing any trial procedure, the signed informed consent form needs to be obtained (Day 1 [M0] unless obtained at Screening). Refer to Section 9.1.1.
2. Check inclusion and exclusion criteria (Day 1 [M0]). Refer to Section 7.1 and Section 7.2, respectively.
3. Assign subject to trial group (Day 1 [M0]). Refer to Section 6.1.
4. Collect demographic data, medical history, and prior medication/vaccination (Day 1 [M0]). Refer to Section 9.1.2.
5. Collect concomitant medications/vaccinations. Refer to Section 9.1.2.
6. Review of systems: Review of systems is a structured interview that queries the subject as to any complaints the subject has experienced across each organ system.
7. Perform a complete physical examination. Refer to Section 9.1.4.
8. Check vital signs. Refer to Section 9.1.5.
9. Perform pregnancy testing (serum or urine) for females of childbearing potential. Refer to Section 9.1.11.

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10. Provide guidance with respect to the avoidance of pregnancy for females of childbearing potential who are sexually active. Refer to Section 9.1.11.
11. Collect pre-vaccination blood samples. Refer to Sections 9.1.7, 9.1.8, and 9.1.10.

    Blood should be taken from the subject using an aseptic venipuncture technique.

9.3.3 Vaccination Procedures (Day 1 [M0] and Day 90 [M3])

1. Check criteria for delay of trial vaccine administration. Refer to Section 7.3.
2. Check contraindications for trial vaccine administration. Refer to Section 7.5.
3. Administer the trial vaccine. Refer to Section 8.1.3.

9.3.4 Post Vaccination Procedures (Day 1 [M0] and Day 90 [M3])

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

Diary card instructions must include the following:

- The individual(s) who will enter the information into the diary card must understand that timely completion of the diary card on a daily basis is a critical component of trial participation. This individual should also be instructed to write clearly and to complete the diary card in pen. Any corrections to the diary card that are performed by the individual(s) completing the diary card should include a single strikethrough line with a brief explanation for any change and be initialed and dated.

Please note:

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

- The diary card should be reviewed with the subject.
- No corrections or additions to the diary card will be allowed after it is reviewed with the investigator/designee.

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- Any data that is identified as implausible or incorrect, and confirmed by the subject to be a transcription error, should be corrected by the subject on the diary card (the correction should include a single strikethrough line and should be initialed and dated by the subject).
- Any blank or illegible fields on the diary card not otherwise corrected as above will be missing in the eCRF.
- The site must enter all readable entries on the diary card into the eCRF.
- Any newly described solicited and unsolicited (non-serious) safety information should be added to the diary card by the subject, initialed, and dated.
- Starting on the day of vaccination (Day 1 [M0] and Day 90 [M3]), the subject will check for specific types of events at the injection site (ie, solicited local [injection site] reactions), any specific generalized symptoms (solicited systemic events), body temperature, unsolicited (non-serious) AEs, any other symptoms or change in the subject's health status, any medications taken (excluding vitamins and minerals), and any vaccination. Solicited local (injection site) reactions, solicited systemic events, body temperature, and unsolicited (non-serious) AEs will be recorded in the diary. Assessments should preferably take place in the evening at day's end.
- Body temperature measurement is to be performed using the thermometer provided by the site. Assessments should take place at approximately the same time of day, preferably in the evening at day's end. If the subject feels unusually hot or cold during the day, the subject should check their temperature. If the subject has fever, the highest body temperature observed that day should be recorded on the diary card.
- The measurements of solicited local (injection site) reactions are to be performed using the ruler provided by the site.
- The collection on the diary card of solicited local (injection site) reactions, solicited systemic events (including body temperature measurement), and unsolicited (non-serious) AEs will continue for a total of 7 days, 14 days, and 28 days; respectively, following administration of each trial vaccine dose (including the day of administration). Any solicited local (injection site) reaction or solicited systemic event observed as continuing on Day 8 or 15, respectively, following each trial vaccination will be recorded as an AE on the "Adverse Event" eCRF for follow-up. Any solicited local (injection site) reaction or systemic event that resolves before 8 or 15 days, respectively, following each trial vaccination, but recurs at a later time (ie, if discontinues), should be recorded as an unsolicited AE on the "Adverse Event" eCRF (see Section 10.4.1 and Section 10.4.2).
- Collect and record MAAEs. Refer to Section 10.4.3.
- Collect and report SAEs. Refer to Section 10.4.4.

After each trial vaccination, the subject will be observed for at least 30 minutes including observation for solicited local (injection site) reactions, solicited systemic events (including body

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temperature measurement), and unsolicited AEs. Information should be recorded in the eCRF. The investigator or delegate will take the opportunity to remind the subject how to measure solicited local (injection site) reactions and body temperature as part of this observation period. All safety data will be collected in the subject's source documents.

The site should schedule the next trial visit with the subject.

The subject will receive a written reminder of the next trial visit.

The subject will be reminded to complete the diary card daily, to contact the site if there are any questions, and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious. All contact details will be provided to the subject.

9.3.5 Site Visits after Vaccination (Post First Vaccination: Days 4, 6, 9, 12, 15, 30 [M1], and 60 [M2]; Post Second Vaccination: Days 94, 96, 99, 102, 105, 120 [M4], 150 [M5], 180 [M6], 270 [M9], and 360 [M12])

Note: Refer to Section 2.1 for visit windows(days) between trial visits that do NOT include a vaccination.

1. Review the diary card with the subject (Days 4, 6, 9, 12, 15, 30 [M1], 94, 96, 99, 102, 105, and 120 [M4]). Refer to Section 9.3.4.
2. Collect and record persistent/prolonged solicited local (injection site) reactions (Days 9, 12, 15, 30 [M1], 60 [M2], 99, 102, 105, 120 [M4], 150 [M5], 180 [M6], 270 [M9], and 360 [M12]). Refer to Section 9.3.4 and Section 10.4.2.
3. Collect and record persistent/prolonged solicited systemic events (Days 15, 30[M1], 60 [M2], 120 [M4], 150 [M5], 180 [M6], 270 [M9], and 360 [M12]). Refer to Section 9.3.4 and Section 10.4.2.
4. Collect concomitant medications/vaccinations. Refer to Section 9.1.2.
5. Perform a targeted physical examination (Days 30 [M1], 120 [M4], and 360 [M12]). Refer to Section 9.1.4 and Section 9.1.5.
6. Check vital signs. Refer to Section 9.1.5.
7. Provide guidance with respect to the avoidance of pregnancy for females of childbearing potential who are sexually active (Days 4, 6, 9, 12, 15, 30 [M1], 60 [M2], 94, 96, 99, 102, 105, and 120 [M4]). Refer to Section 9.1.11.
8. Collect and record MAAEs. Refer to Section 10.4.3.
9. Collect and report SAEs. Refer to Section 10.4.4.
10. Collect blood samples. Refer to Section 9.1.7 and Section 9.1.10.

    Blood should be taken from the subject using an aseptic venipuncture technique.

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The investigator or delegate should schedule the next trial visit with the subject, as applicable.

The subject will receive a written reminder of the next trial visit, as applicable. The subject will be reminded to complete the diary card daily (Days 4, 6, 9, 12, 15, 94, 96, 99, 102, and 105), to contact the site if there are any questions, and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious. All contact details will be provided to the subject.

9.3.6 Phone Contacts - Reminder Calls

Not applicable.

9.3.7 Phone Contacts – Safety Call

When a site visit cannot be carried out due to the COVID-19 pandemic, telephone contacts will be made for subjects who are still under monitoring for safety reporting. Refer also to Section 14.1.

9.3.8 Final (End of Trial) Visit

The final (end of trial) visit will be performed on Day 360 (M12). If a subject terminates earlier, the final (end of trial) visit procedures should be performed at their last trial visit, if possible. For all subjects receiving trial vaccine, the investigator must complete the "End of Trial" eCRF.

9.3.9 Post-Trial Care

No post-trial care will be provided.

9.4 Biological Sample Retention and Destruction

In this trial, specimens will be collected for serological testing as described in Section 9.1.6, immune response testing as described in Section 9.1.7. and Section 9.1.8, and for clinical safety as described in Section 9.1.10. All blood samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guideline provided to the site. Samples will be preserved and retained at a central laboratory that was contracted by the sponsor for this purpose for up to but no longer than 20 years or as required by applicable law. The sponsor has put into place a system to protect the subject's personal information to ensure optimal confidentiality and defined standard processes for sample and data collection, storage, analysis, and destruction.

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

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10.0 ADVERSE EVENTS

10.1 Definitions

10.1.1 Adverse Events

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

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

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

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

10.1.2 Solicited Adverse Events

The occurrence of selected indicators of safety will be measured/collected for 7 days (solicited local [injection site] reactions) and 14 days (solicited systemic events) following administration of each trial vaccine dose (including the day of administration) and will be recorded on the "Local and Systemic Reactions" eCRF, as applicable, and as listed in Table 10.a.

Any solicited local (injection site) reaction or systemic event observed as continuing on Day 8 or Day 15, respectively, following each trial vaccination will be recorded as an AE on the "Adverse Event" eCRF for follow-up. For these persistent/prolonged solicited AEs, the end date will be captured on the "Adverse Event" eCRF to permit a separate analysis from the unsolicited AEs (see Section 10.4.1 and Section 10.4.2).

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Table 10.a Solicited Local (Injection Site) Reactions and Systemic Events

Local (injection site) reactions:	Pain
	Erythema
	Swelling
Systemic events:	Fever [a]
	Headache
	Asthenia
	Malaise
	Myalgia

(a) Fever is defined as body temperature greater than or equal to 38°C (100.4°F) regardless of method taken [19].

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

Table 10.b Severity Scales for Solicited Safety Parameters

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

(a) Subjects are to record greatest surface diameter in mm on the diary card.
(b) Fever is defined as body temperature greater than or equal to 38°C (100.4°F) regardless of method taken [19].

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10.1.3 Adverse Events of Special Interest

Not applicable.

10.1.4 Medically Attended Adverse Events

MAAEs are defined as AEs leading to an unscheduled medical visit to or by a healthcare professional including visits to an emergency department, but not fulfilling seriousness criteria.

10.1.5 Serious Adverse Events

An SAE is defined as any untoward medical occurrence that:

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

10.2 Causality of Adverse Events

Relationship (causality) to the trial vaccine will also be assessed by the investigator. The relationship of each AE to the trial vaccine, including solicited systemic events (solicited local [injection site] reactions are considered as related by default) will be assessed using the following categories:

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

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10.2.1 Relationship to Trial Procedures

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

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

10.2.2 Outcome of Adverse Events

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

10.3 Additional Points to Consider for Adverse Events

An untoward occurrence generally may:

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

Diagnoses vs signs and symptoms:

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

Worsening of AEs:

- If the subject experiences a worsening or complication of an AE after administration of the trial vaccine, the worsening or complication should be recorded as a new AE. Investigators

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should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

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

Changes in severity of AEs:

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

Preplanned surgeries or procedures:

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

Elective surgeries or procedures:

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

Trial procedures:

- Adverse occurrences related to trial procedures after signing of the informed consent form are considered as AEs and should be reported as AEs.

10.4 Procedures

10.4.1 Collection and Reporting of Adverse Events

All AEs, whether considered related to the use of the trial vaccine or not, must be monitored until symptoms subside and any abnormal laboratory values have returned to Baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full autopsy report should be supplied, if possible. All findings must be reported on the "Adverse Event" eCRF and on the SAE form*, if necessary (see Section 10.4.4). All findings in subjects experiencing AEs must also be documented in the subject's source documents. Any unsolicited AEs will be collected on diary cards by the subject for 28 days following administration of each trial vaccine dose (including the day of administration). AEs leading to discontinuation (from the trial or from the vaccination regimen) are collected throughout the trial. Even if the subject is deemed ineligible to receive further doses of trial vaccine, all efforts should be made to continue the collection of safety data according to protocol.

- Reported term for the AE.
- Start and end date.

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- Serious (Yes/No).
- Severity.
- Investigator's opinion of the causality (relationship) between the event and administration of trial vaccine ("related" or "not related").
- Investigator's opinion of the causality (relationship) to trial procedure(s), including the details of the suspected procedure.
- Action taken with the trial vaccine.
- Outcome of event.

**SAE reporting will be done by eCRF. If the eCRF system is unavailable, a paper sponsor SAE form/paper CRF should be completed and the event must be entered into the eCRF once access is restored.*

10.4.2 Collection and Reporting of Solicited Adverse Events

The occurrence of selected indicators of safety will be collected on diary cards by the subject for 7 days (solicited local [injection site] reactions) and 14 days (solicited systemic events) following administration of each trial vaccine dose (including the day of administration) and will be recorded on the "Local and Systemic Reactions" eCRF, as applicable. These will be summarized in the final report under the category "solicited AEs" to differentiate them from unsolicited AEs.

Any solicited local (injection site) reaction or solicited systemic event observed as continuing on Day 8 or 15, respectively, following each trial vaccination will be recorded as an AE on the "Adverse Event" eCRF for follow-up. For these persistent/prolonged solicited AEs, the end date will be captured on the "Adverse Event" eCRF to permit a separate analysis from the unsolicited AEs. Any solicited local (injection site) reaction or systemic event that resolves before 8 or 15 days, respectively, following each trial vaccination, but recurs at a later time (ie, if discontinues), should be recorded as an unsolicited AE on the "Adverse Event" eCRF (see Section 10.4.1).

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

- Solicited local (injection site) reactions or systemic events that lead the subject to withdraw from the trial.
- Solicited local (injection site) reactions or systemic events that lead to the subject being withdrawn from the trial by the investigator.
- Solicited local (injection site) reactions and systemic events that otherwise meet the definition of an SAE (see Section 10.1.5).

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10.4.3 Collection and Reporting of Adverse Events of Special Interest/Medically Attended Adverse Events

AEs of special interest will not be collected for this trial.

MAAEs occurring from first trial vaccination at Day 1 (M0) until the end of the trial (Day 360 [M12]) will be collected during site visits by interview and must be recorded as AEs on the "Adverse Event" eCRF.

10.4.4 Collection and Reporting of Serious Adverse Events

Collection of SAEs will commence from the time that the subject is first administered the trial vaccine (Day 1 [M0]). Routine collection of SAEs will continue until the end of the trial (Day 360 [M12]).

SAEs should be reported according to the following procedure:

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

- A short description of the event and the reason why the event is categorized as serious.
- Causality assessment.
- Protocol number.
- Subject identification number.
- Investigator's name.
- Name of trial vaccine.

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

10.5 Follow-up Procedures

10.5.1 Adverse Events

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

10.5.2 Serious Adverse Events

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

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All SAEs should be followed up until resolution, permanent outcome of the event, or is otherwise explained. The timelines and procedure for follow-up reports are the same as those for the initial report.

10.5.3 Safety Reporting to Investigators, Investigational Review Boards or Independent Ethics Committees, and Regulatory Authorities

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

10.5.4 Post-Trial Events

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

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11.0 TRIAL-SPECIFIC REQUIREMENT(S)

11.1 Trial-Specific Committees

11.1.1 Data Monitoring Committee

A DMC will have oversight of this trial. The DMC functions at a program level and further information is available in the DMC Charter.

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12.0 DATA HANDLING AND RECORD KEEPING

The full details of procedures for data handling will be documented in the Data Management Plan. AEs, medical history, and concurrent medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Drugs will be coded using the WHO Drug Dictionary [20].

12.1 CRFs (Electronic)

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

The sponsor or designee will supply the investigative site with access to eCRFs. The sponsor will make arrangements to train appropriate site staff in the use of the eCRF. Electronic CRFs must be completed in English.

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

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

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

When a site visit cannot be carried out due to the COVID-19 pandemic, telephone contacts will be made for subjects for whom monitoring for safety reporting is still ongoing. Refer also to Section 14.1.

12.2 Record Retention

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

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

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

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13.0 STATISTICAL METHODS

13.1 Statistical and Analytical Plans

A Statistical Analysis Plan (SAP) will be prepared and finalized prior to the database lock. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all trial objectives.

Data reviews will be conducted prior to the database lock. These reviews will assess the accuracy and completeness of the trial database and subject evaluability.

This trial is designed for a descriptive evaluation of the endpoints that does not require hypotheses testing. Further details will be provided in the SAP.

Summaries will be provided by trial group (Group 1 and Group 2) and further for Group 2 by subgroups (DENV-1 or DENV-3 seropositive).

As trial laboratory procedures are not carried out during the COVID-19 pandemic there is a possibility that not all pre-defined test results will be available prior to database lock. Thus, there is a risk for database unlock/relock and additional data may be added in CSR amendments or addenda to the final CSR.

13.1.1 Analysis Sets

Safety Set: The Safety Set will consist of all subjects who received at least 1 dose of trial vaccine.

Full Analysis Set (FAS): The FAS will include all subjects who received at least 1 dose of trial vaccine and for whom a valid pre-dose and at least one valid post-dose blood sample is taken.

Per–Protocol Set (PPS): The PPS will include all subjects in the FAS who have no major protocol violations. The major protocol violation criteria will be defined as part of the data review prior to database lock. The categories of major protocol violations include: (1) not meeting selected entry criteria, (2) receiving prohibited therapies, (3) not receiving 2 doses of TDV, or receiving the second vaccination inadmissibly outside of the visit window, and (4) other major protocol violations that may be identified during data review prior to database lock.

13.1.2 Analysis of Demographics and Other Baseline Characteristics

Age, gender, race, and other Baseline characteristics will be summarized descriptively.

Summaries will be provided by trial group (Group 1 and Group 2) and further for Group 2 by subgroups (DENV-1 or DENV-3 seropositive). Unless specified otherwise, number of subjects with non-missing observations, mean, SD, median, minimum and maximum will be presented for continuous data; frequency and percent will be presented for categorical data.

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13.1.3 Immunogenicity Analysis

For the primary immunogenicity endpoint, descriptive statistics and 95% CIs will be provided for each applicable visit. Summaries will be provided by trial group (Group 1 and Group 2) and further for Group 2 by subgroups (DENV-1 or DENV-3 seropositive).

The primary immunogenicity analyses will be based on the PPS; supportive analyses may be provided based on the FAS.

Similar descriptive analyses as for the primary immunogenicity endpoint will be provided for the secondary and exploratory endpoints for each applicable assay at all relevant time points, based on the PPS. Supportive analyses based on the FAS may also be provided for selected endpoints.

Antibody titers will be analyzed under log-normal distribution assumption. GMT will be calculated for each relevant time point as anti-logarithm of arithmetic mean of natural log transformed titers. The 95% CI for GMT will be calculated as the anti-log transformation of upper and lower limits for a 2-sided CI of the mean of the log-transformed titers (based on Student's t-distribution).

Other quantitative data will be summarized descriptively on the original scale.

Seropositivity rates will be accompanied with 95% CIs calculated by exact (Clopper-Pearson) method [21].

Relationship between immunogenicity measures will be assessed using non-parametric correlation coefficient, as appropriate [22]. Graphical methods will also be explored. Further details will be provided in the SAP.

Handling of missing data will be described in the SAP.

13.1.4 Safety Analysis

All safety data will be summarized descriptively using the Safety Set, by trial group (Group 1 and Group 2) and further for Group 2 by subgroups (DENV-1 or DENV-3 seropositive).

Solicited AEs

For each solicited AE, the number and percentage of subjects with local (injection site) reactions and systemic events will be summarized by event severity for each day after each vaccination (ie, Day 1 [M0] through Day 7 for local [injection site] reactions and Day 1 [M0] through Day 14 for systemic events), and overall. Summaries of first onset of each event and the number of days subjects reported experiencing each event will also be provided. For subjects with more than 1 episode of the same event, the maximum severity will be used for tabulations.

Persistent/prolonged solicited local (injection site) reactions or systemic events continuing on Day 8 or Day 15, respectively, following each trial vaccination will be assessed separately. Unless otherwise specified, these events will not be included in the analyses/tabulations of unsolicited AEs and will have separate listings.

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Unsolicited AEs

Unsolicited AEs will be coded using MedDRA, and summarized by System Organ Class (SOC) and Preferred Term (PT). Unsolicited AEs will be summarized for 28 days following each vaccination (day of vaccination + 27 days) as follows: by PT including events with frequency greater than a pre-defined threshold (the percentage will be specified in the SAP); by SOC and PT; by SOC, PT, and severity; and by SOC, PT, and relationship to TDV. Subjects reporting more than 1 occurrence for the term (level) being summarized will be counted only once.

AEs leading to trial vaccine withdrawal or trial discontinuation will be collected and summarized for the entire trial period.

SAEs and MAAEs

SAEs and MAAEs will be coded using MedDRA, and summarized by SOC and PT for the entire trial period.

Other Safety Observations

Vaccine viremia (incidence, duration, and level) will be evaluated descriptively at all applicable time points.

Relationship between vaccine viremia and immunogenicity measures will be assessed using non-parametric correlation coefficient, as appropriate [21]. Graphical methods will also be explored. Further details will be provided in the SAP.

13.2 Interim Analysis and Criteria for Early Termination

No interim analysis is planned.

13.3 Determination of Sample Size

This trial is designed for a descriptive evaluation of the endpoints that does not require hypotheses testing. Therefore, the sample size was not determined based on formal statistical power calculations.

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14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Trial-Site Monitoring Visits

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

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

In the event a monitor cannot visit the site in a timely manner due to the COVID-19 pandemic, alternative monitoring approaches such as remote source data verification (SDV) or telephone contact may be used to ensure data quality and integrity and maintain subject safety. Alternative monitoring approaches should be used only where allowed by the local Health Authority and when approved by the IRB/IEC. During remote monitoring, the monitor should focus on trial activities that are essential to the safety of trial subjects and/or data reliability.

14.2 Protocol Deviations

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

14.3 Quality Assurance Audits and Regulatory Agency Inspections

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

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14.4 Trial Risk Management

The ICH E6 addendum (R2) guidance encourages a risk-based approach to the management of clinical trials and includes requirements for risk control and risk reporting. Takeda or designee established Quality Tolerance Limits (QTL) taking into consideration the medical and statistical characteristics of the variables and the statistical design of the trial. This process was performed according to Takeda internal procedures.

At the end of the trial, the quality management approach implemented will be described in the CSR. If applicable, the CSR will summarize important deviations from the predefined QTL and the remedial actions taken.

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15.0 ETHICAL ASPECTS OF THE TRIAL

This trial will be conducted with the highest respect for the trial subjects according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki [1], and the ICH Harmonized Tripartite Guideline for GCP [2]. Each investigator will conduct the trial according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the "Responsibilities of the Investigator" that are listed in Appendix A. The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

15.1 Institutional Review Board and/or Independent Ethics Committee Approval

IRBs and IECs must be constituted according to the applicable federal requirements of each participating region. The sponsor or designee will require documentation noting all names and titles of members who make up the respective IRB or IEC. If any member of the IRB or IEC has direct participation in this trial, written notification regarding his or her abstinence from voting must also be obtained. If the US site is unwilling to provide names and titles of all members due to privacy and conflict of interest concerns, the site should instead provide a Federal Wide Assurance Number or comparable number assigned by the Department of Health and Human Services.

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

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

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

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15.2 Subject Information, Informed Consent, and Subject Authorization

Written consent documents will embody the elements of informed consent as described in the Declaration of Helsinki [1] and the ICH Guidelines for GCP [2] and will be in accordance with all applicable laws and regulations. The informed consent form, subject authorization form (if applicable), and subject information sheet describe the planned and permitted uses, transfers, and disclosures of the subject's personal and personal health information for the purpose of conducting the trial. The informed consent form and the subject information sheet further explain the nature of the trial, its objectives, and potential risks and benefits, as well as the date informed consent is given. The informed consent form will detail the requirements of the subject and the fact that the subject is free to withdraw at any time without giving a reason and without prejudice to the subject's further medical care.

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

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

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

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

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

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15.3 Subject Confidentiality

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

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the sponsor requires the investigator to permit its monitor or designee, representatives from any regulatory authority (eg, FDA, MHRA, PMDA), the sponsor's designated auditors, and the appropriate IRBs and IECs to review the subject's original medical records (source data or documents), including, but not limited to, laboratory test result reports, electrocardiogram (ECG) reports, admission and discharge summaries for hospital admissions occurring during a subject's trial participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent form process (see Section 15.2).

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

15.4 Clinical Trial Registration, Publication and Disclosure Policy

15.4.1 Clinical Trial Registration

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

15.4.2 Clinical Trial Results Disclosure

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

Trial completion corresponds to the date on which the final subject was examined or received an intervention for the purposes of final collection of data (Last Subject Last Visit).

15.4.3 Publication of Trial Results

The results of this trial are expected to be published in a scientific journal. It is anticipated that clinical and laboratory co-investigators will participate in authorship. The authorship, order of

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authors and choice of journal will be proposed by the sponsor to the Principal Investigator, to be eventually agreed upon by all authors themselves. The data analysis center for this trial will provide the analyses needed for publication. Information regarding this trial will be posted on ClinicalTrials.gov and/or other registries according to the local requirements.

15.5 Insurance and Compensation for Injury

Each subject in the trial must be insured in accordance with the regulations applicable to the site where the subject is participating. If a local underwriter is required, then the sponsor or sponsor's designee will obtain clinical trial insurance against the risk of injury to clinical trial subjects. Refer to the Clinical Study Site Agreement regarding the sponsor's policy on subject compensation and treatment for injury. If the investigator has questions regarding this policy, he or she should contact the sponsor or sponsor's designee.

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16.0 REFERENCES

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Appendix A Responsibilities of the Investigator

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

The investigator agrees to assume the following responsibilities:

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

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12. Report AEs to the sponsor promptly. In the event of an SAE, notify the sponsor within 24 hours.
13. Review and provide a signature as approval of the content of the CSR.

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Appendix B Investigator Consent to Use of Personal Information

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

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

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

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

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

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Signature Page for DEN-210 Protocol Amendment 4, Version 5.0, 18 December 2020
Title: An Open-Label, Phase 2 Trial to Investigate the Humoral and Cell-Mediated

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