



US011464815B2

(12) **United States Patent**
Wallace

(10) **Patent No.:** US 11,464,815 B2
(45) **Date of Patent:** *Oct. 11, 2022

(54) **DENGUE VACCINE UNIT DOSE AND ADMINISTRATION THEREOF**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **16/295,611**

(22) Filed: **Mar. 7, 2019**

(65) **Prior Publication Data**

US 2020/0069751 A1 Mar. 5, 2020

(30) **Foreign Application Priority Data**

Sep. 5, 2018 (EP)	18192701
Jan. 29, 2019 (EP)	19154334

(51) **Int. Cl.**

<i>A61K 39/12</i>	(2006.01)
<i>A61K 35/76</i>	(2015.01)

(52) **U.S. Cl.**

CPC *A61K 35/76* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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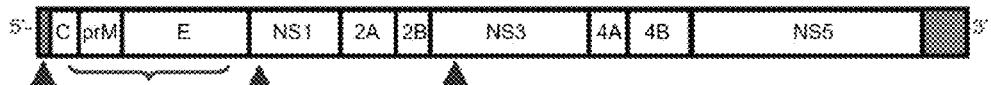
(57) **ABSTRACT**

The invention relates to a unit dose of a dengue vaccine composition and methods and uses for preventing dengue disease and methods for stimulating an immune response to all four dengue virus serotypes in a subject or subject population. The unit dose of a dengue vaccine composition includes constructs of each dengue serotype, such as TDV-1, TDV-2, TDV-3 and TDV-4, at various concentrations in order to improve protection from dengue infection.

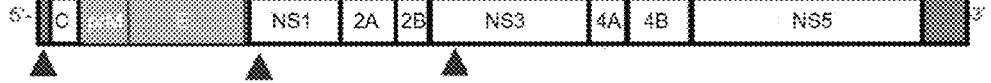
19 Claims, 12 Drawing Sheets

Specification includes a Sequence Listing.

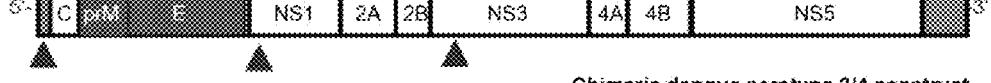
Attenuated dengue serotype 2 construct



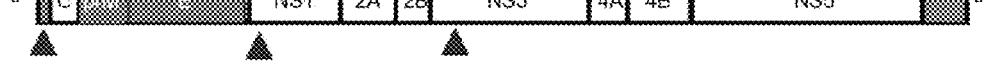
Chimeric dengue serotype 2/1 construct



Chimeric dengue serotype 2/3 construct



Chimeric dengue serotype 2/4 construct



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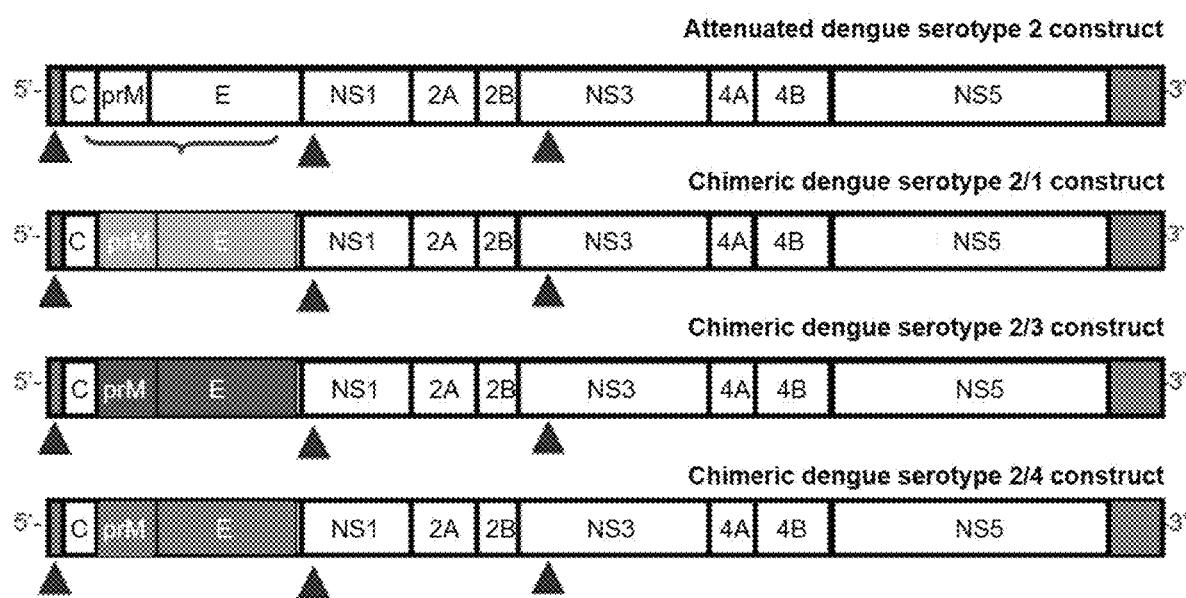


FIG. 1

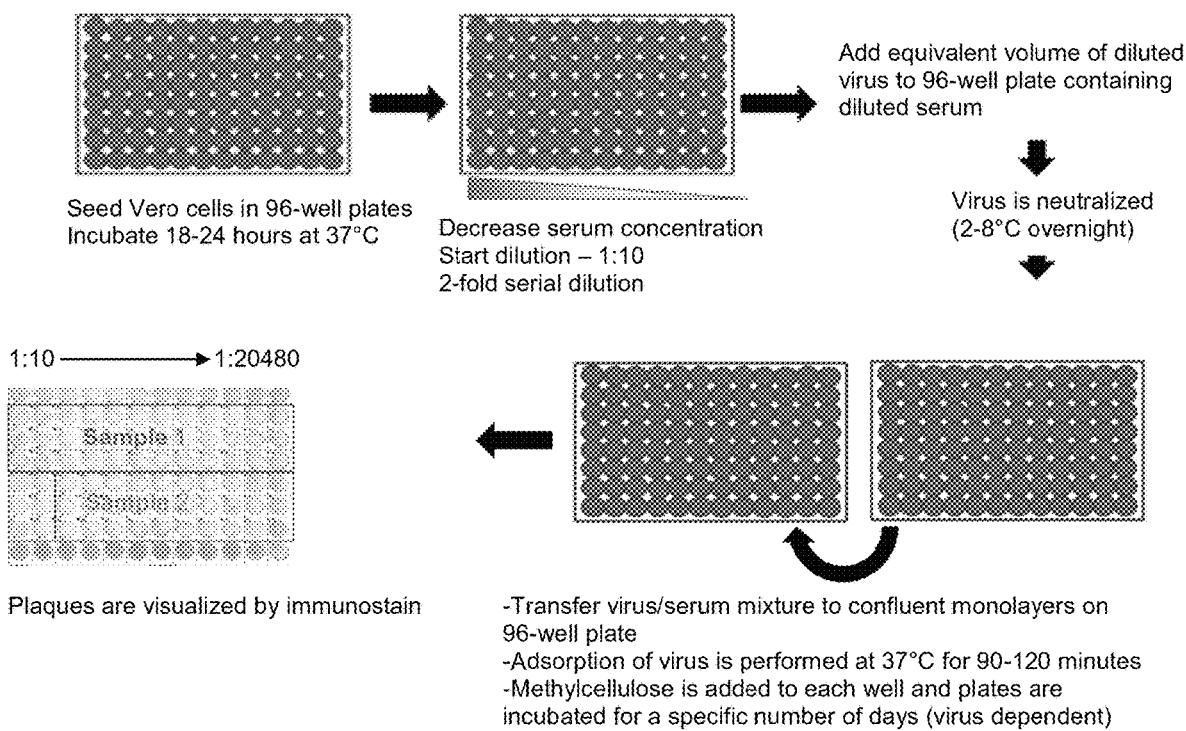


FIG. 2

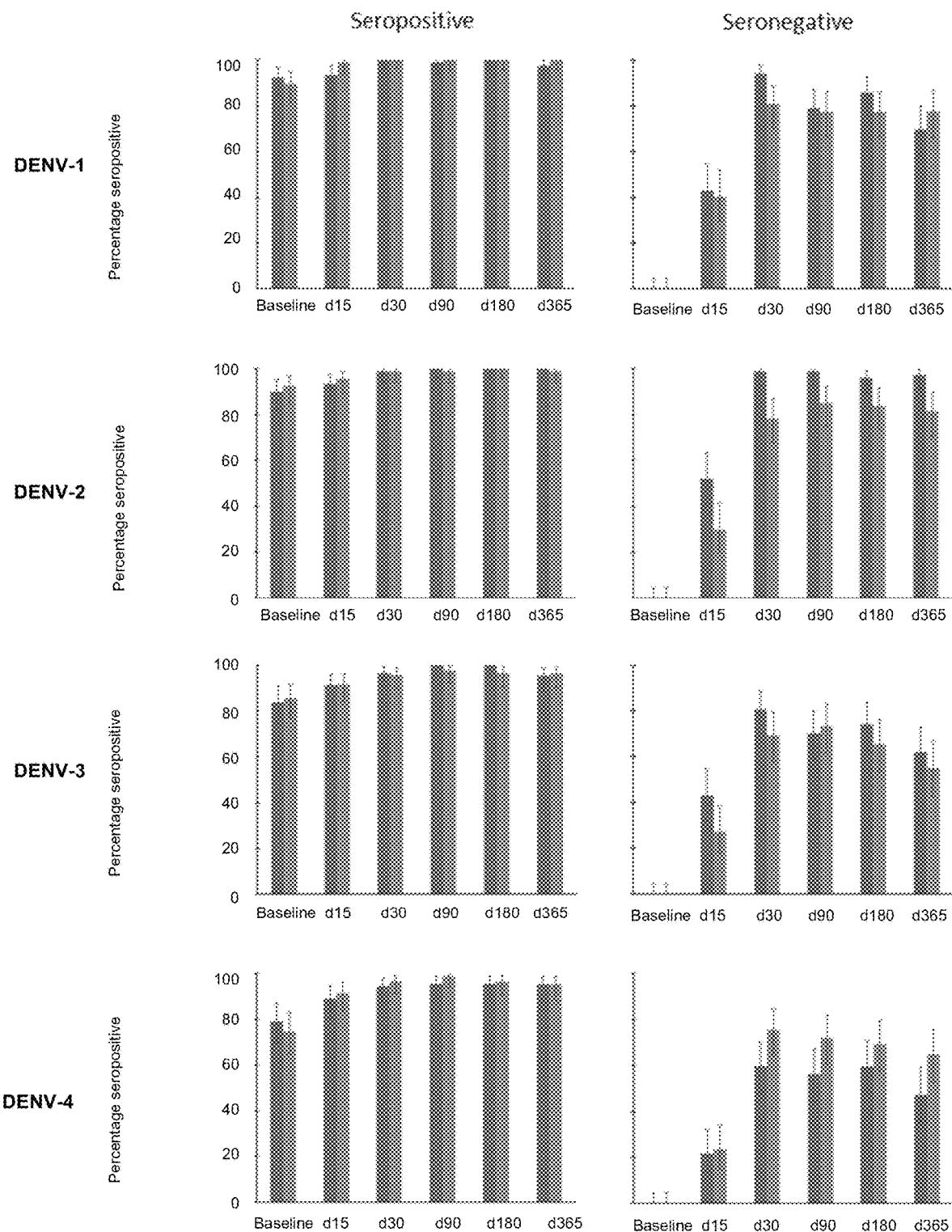
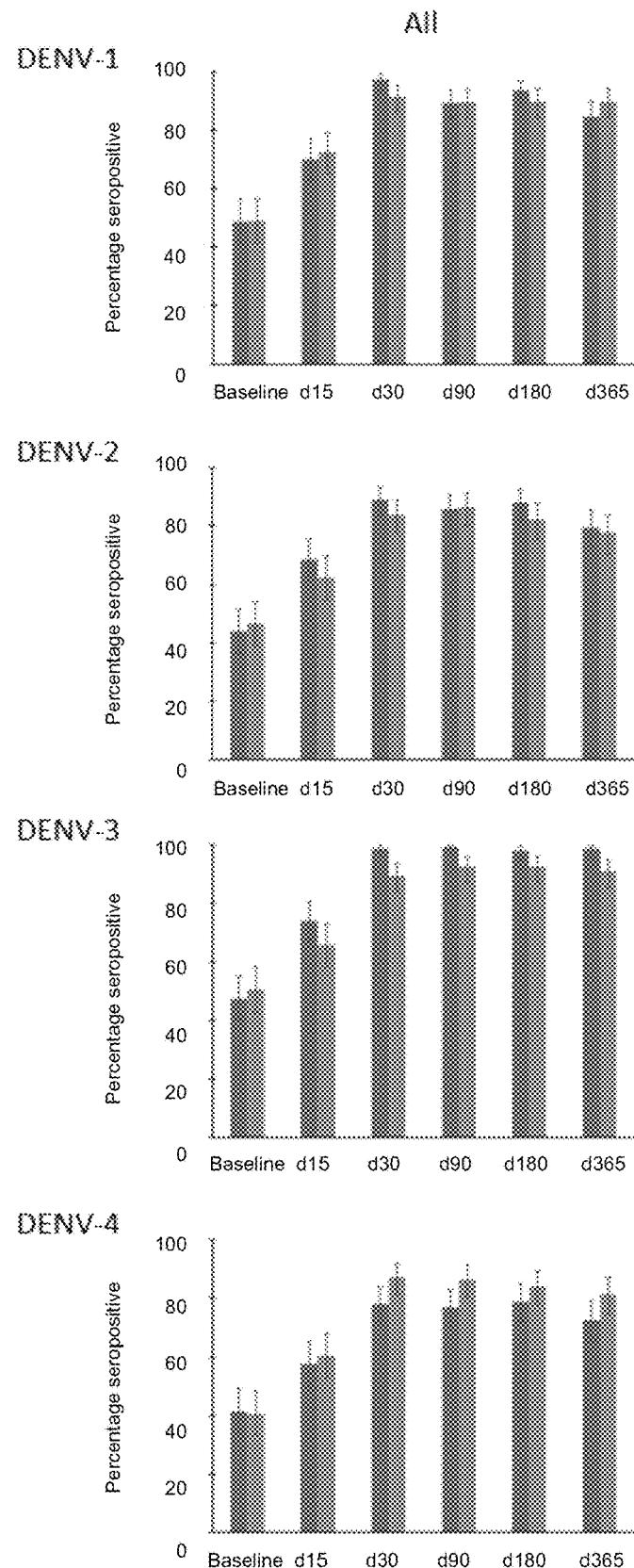


FIG. 3A

**FIG. 3B**

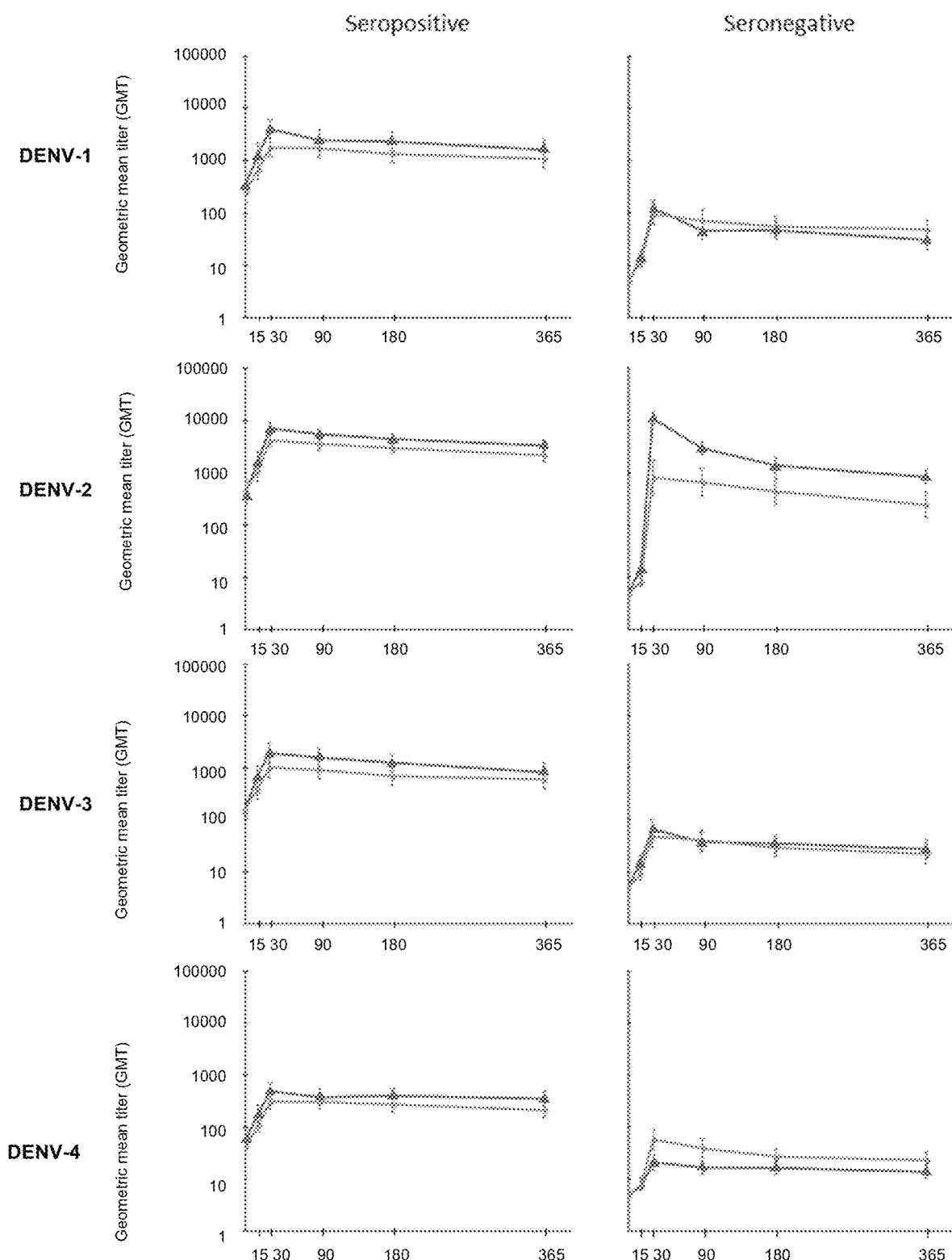
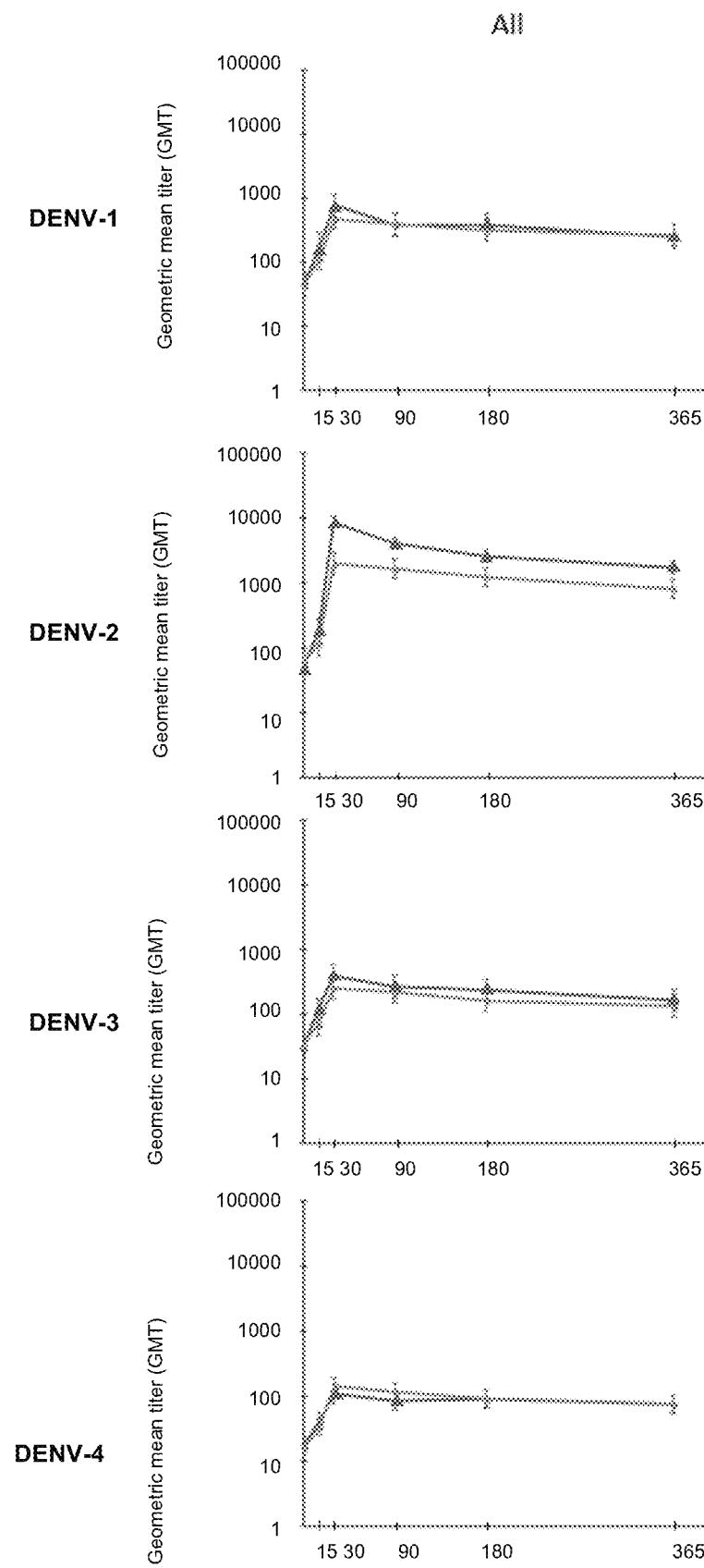


FIG. 4A

**FIG. 4B**

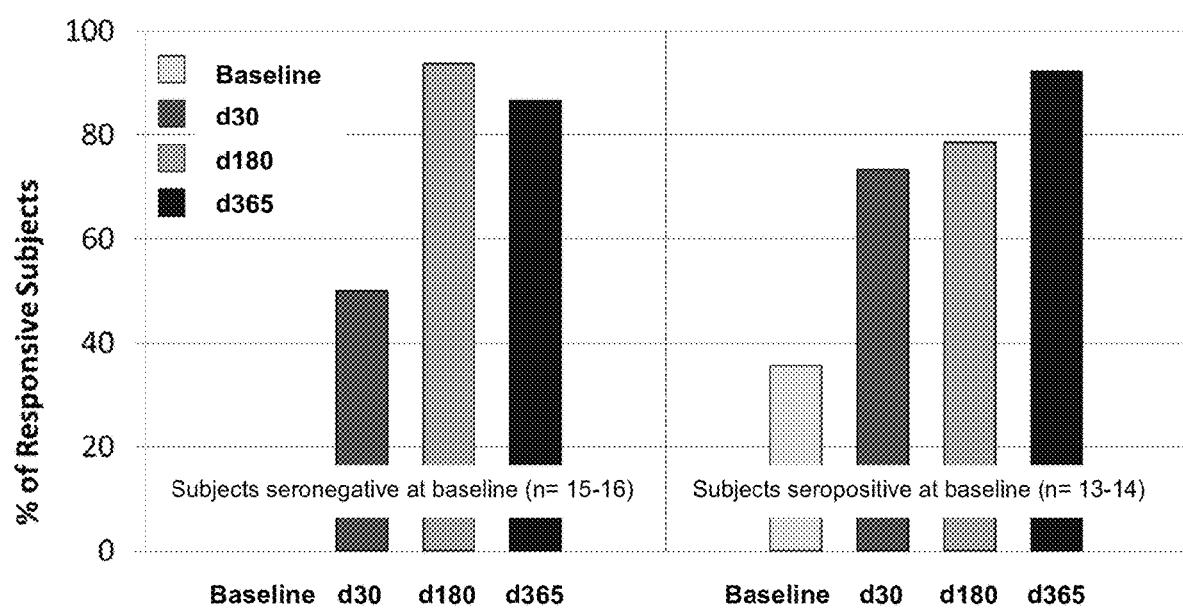


FIG. 5

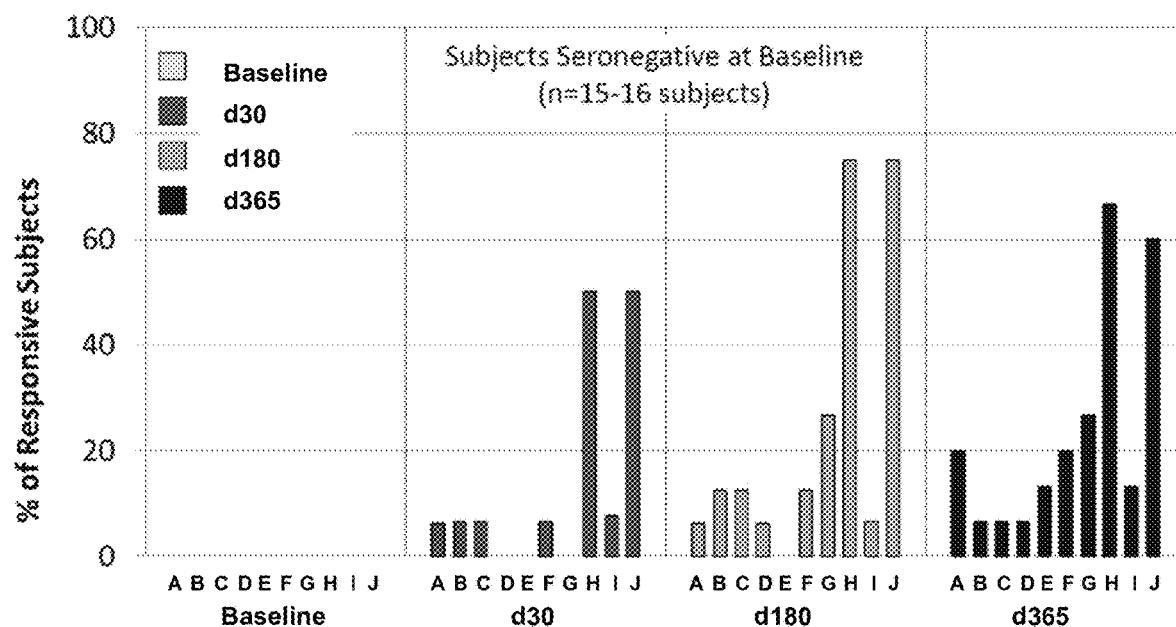


FIG. 6A

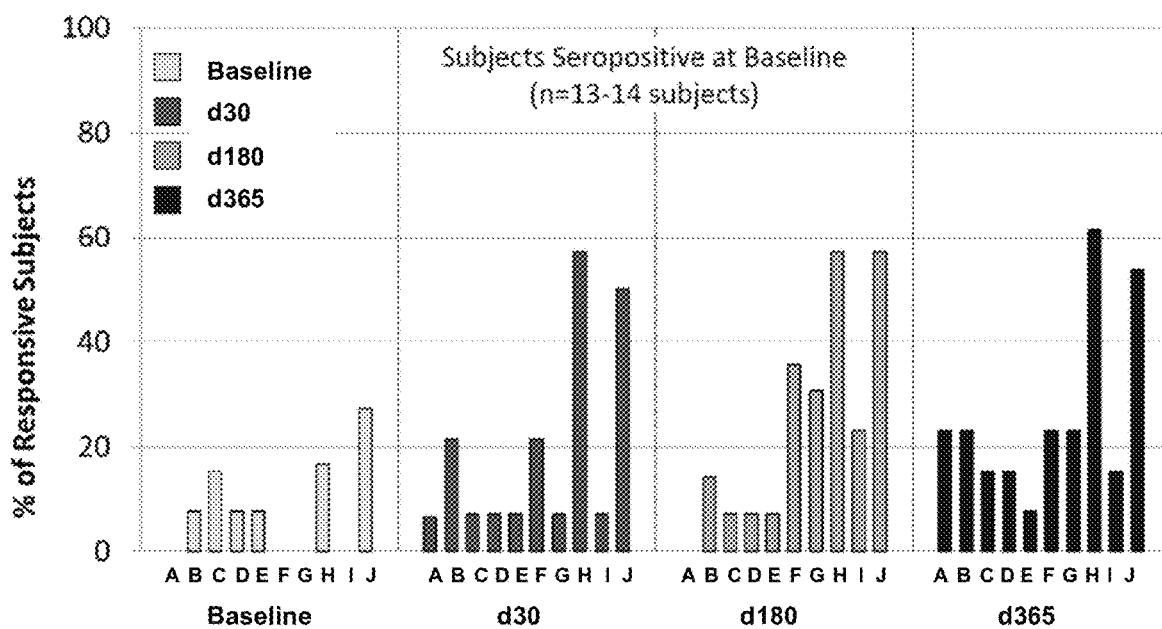
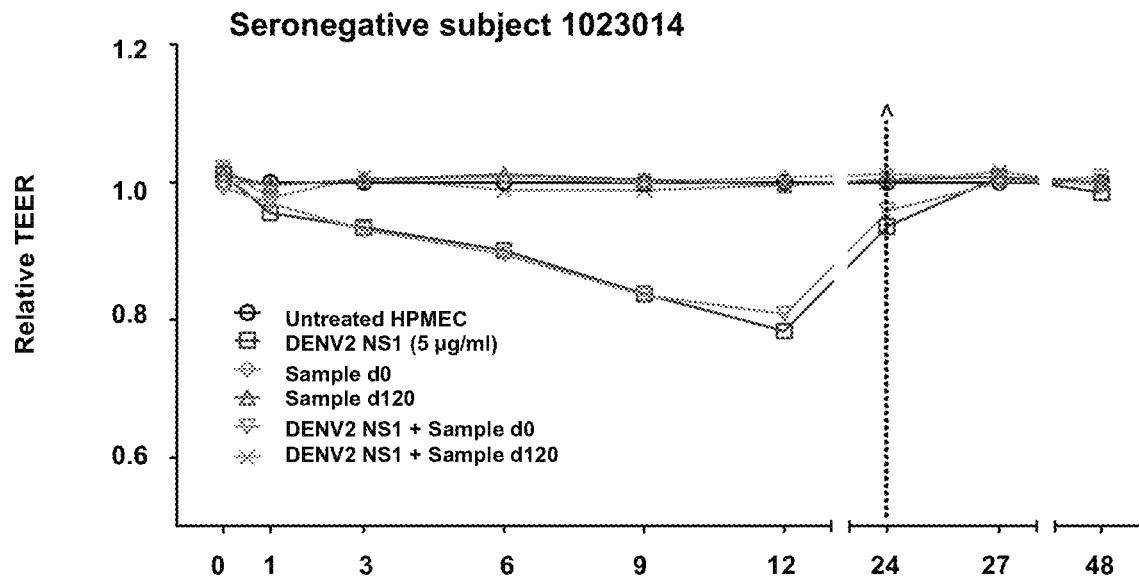
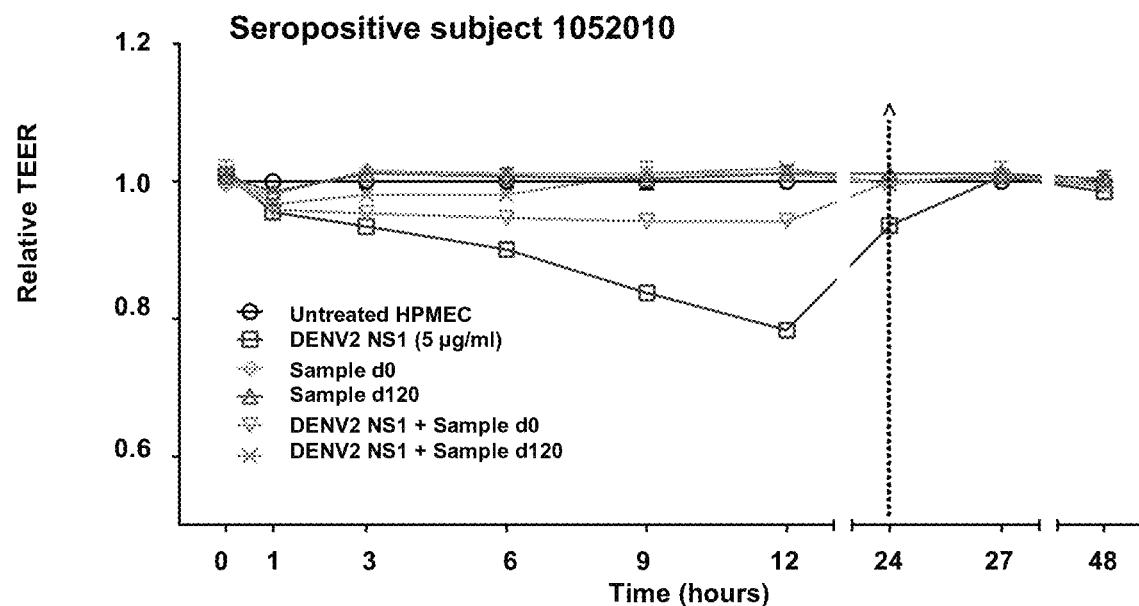
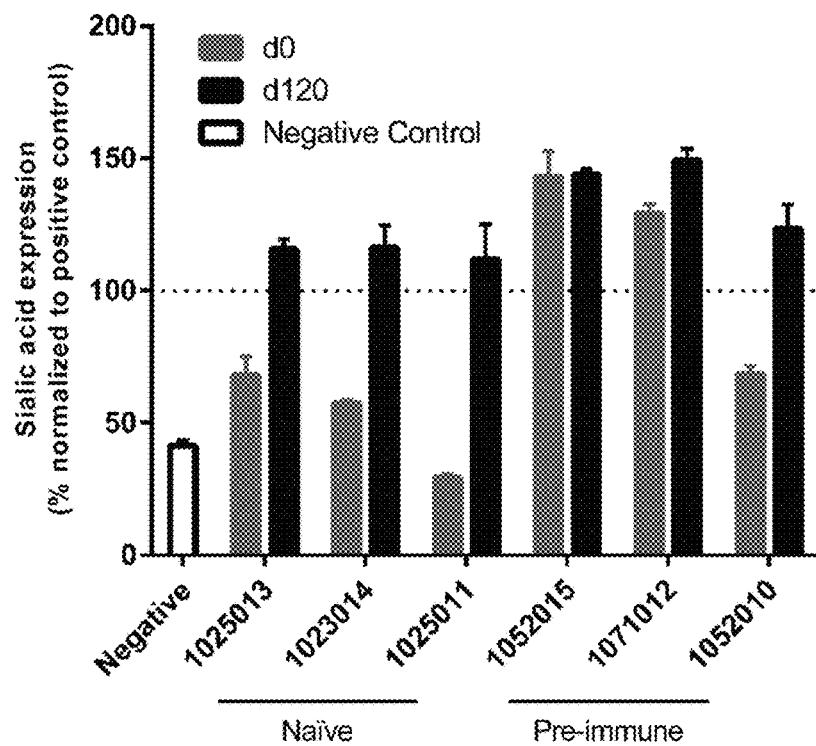
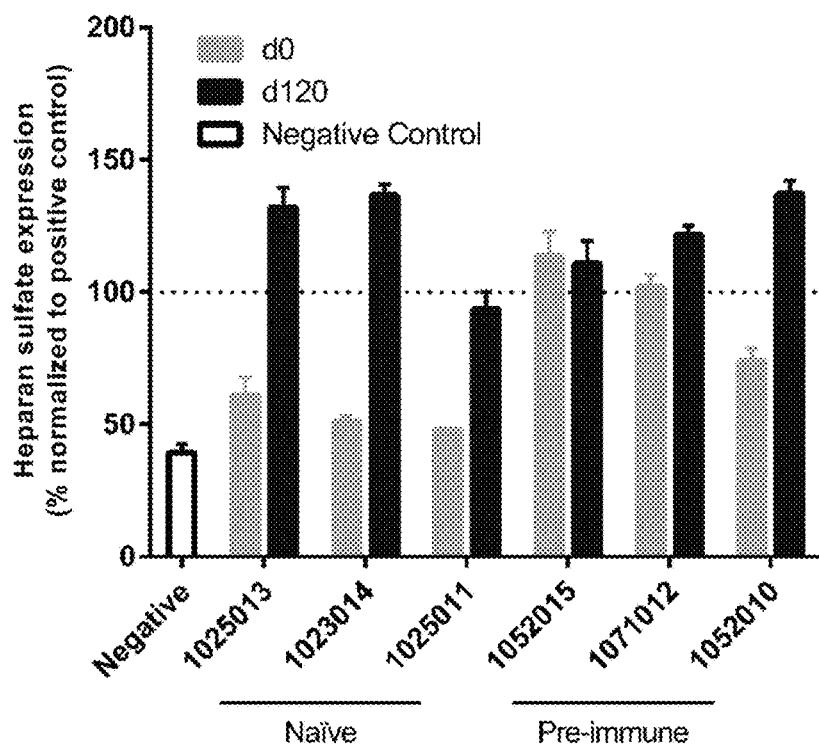


FIG. 6B

**FIG. 7A****FIG. 7B**

**FIG. 8A****FIG. 8B**

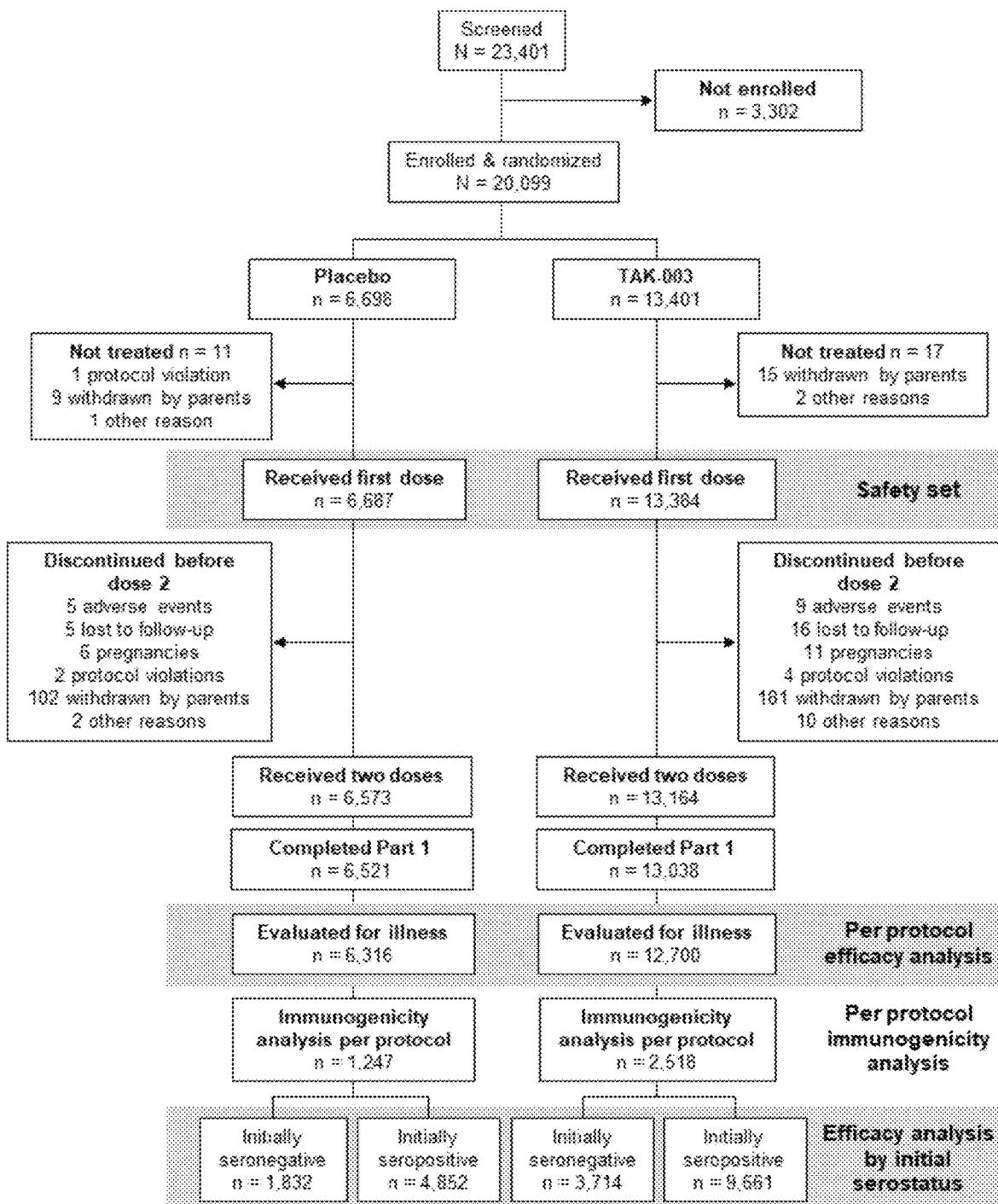
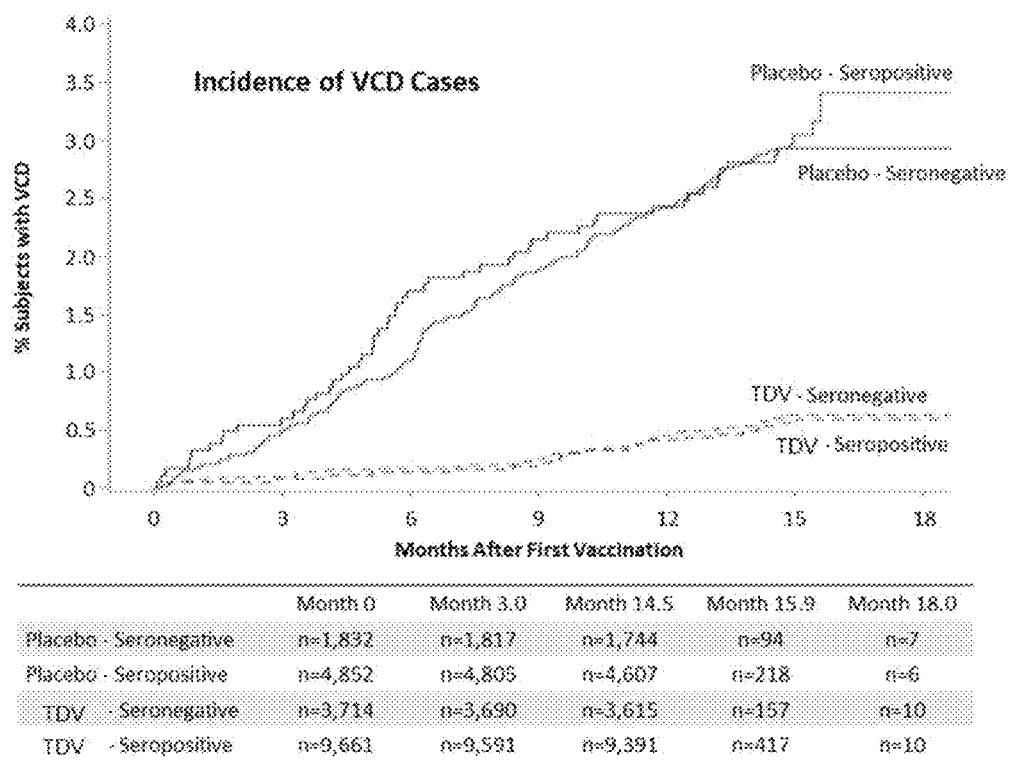
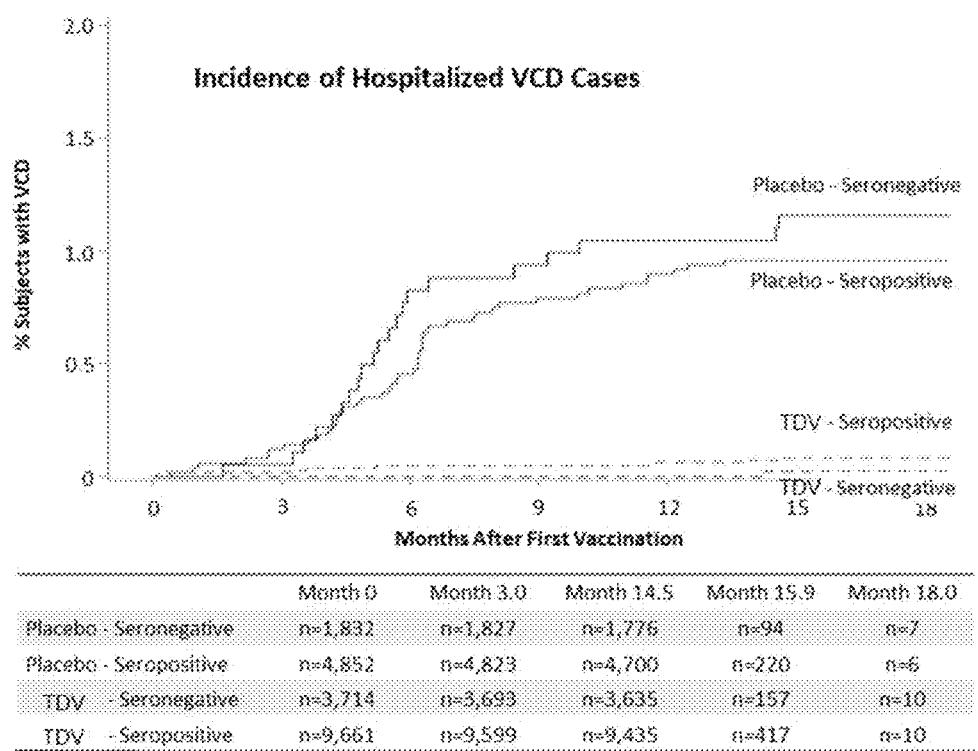


FIG. 9

**FIG. 10A****FIG. 10B**

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DENGUE VACCINE UNIT DOSE AND ADMINISTRATION THEREOF

The Sequence Listing submitted in text format (.txt) filed herewith on Mar. 7, 2019, named "T08269US Sequence Listing" (created on Sep. 5, 2018, 173 KB), is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to unit doses of a dengue vaccine composition and methods for administering a unit dose of a dengue vaccine composition to a subject or a subject population. The unit dose according to this invention provides immune responses against all serotypes of dengue virus, i.e. DENV-1, DENV-2, DENV-3 and DENV-4.

BACKGROUND OF THE INVENTION

Vaccines for protection against viral infections have been effectively used to reduce the incidence of human disease. One of the most successful technologies for viral vaccines is to immunize animals or humans with a weakened or attenuated virus strain (a "live attenuated virus"). Due to limited replication after immunization, the attenuated virus strain does not cause disease. However, the limited viral replication is sufficient to express the full repertoire of viral antigens and can generate potent and long-lasting immune responses to the virus. Thus, upon subsequent exposure to a pathogenic virus strain, the immunized individual is protected from the disease. These live attenuated viral vaccines are among the most successful vaccines used in public health.

Dengue disease is a mosquito-borne disease caused by infection with a dengue virus. Dengue virus infections can lead to debilitating and painful symptoms, including a sudden high fever, headaches, joint and muscle pain, nausea, vomiting and skin rashes. To date, four serotypes of dengue virus have been identified: dengue-1 (DENV-1), dengue-2 (DENV-2), dengue-3 (DENV-3) and dengue-4 (DENV-4). Dengue virus serotypes 1-4 can also cause dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). In the most severe cases, DHF and DSS can be life threatening. Dengue viruses cause 50-100 million cases of debilitating dengue fever, 500,000 cases of DHF/DSS, and more than 20,000 deaths each year, a large portion of which are children. All four dengue virus serotypes are endemic throughout the tropical regions of the world and constitute the most significant mosquito-borne viral threat to humans there. Dengue viruses are transmitted to humans primarily by *Aedes aegypti* mosquitoes, but also by *Aedes albopictus* mosquitoes. Infection with one dengue virus serotype results in life-long protection from re-infection by that serotype, but does not prevent secondary infection by one of the other three dengue virus serotypes. In fact, previous infection with one dengue virus serotype may lead to an increased risk of severe disease (DHF/DSS) upon secondary infection with a different serotype.

To date, only one vaccine, a tetravalent dengue vaccine based on a yellow fever backbone, CYD-TDV (Dengvaxia®, Sanofi Pasteur, Lyon, France), has been licensed in several countries based on the clinical demonstration of an overall vaccine efficacy (VE) against virologically-confirmed dengue (VCD) of 56-61% in children in Asia and Latin America (Capeding M R et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-

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masked, placebo-controlled trial. Lancet 2014, 384:1358-65; Villar L A et al. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9-16 year olds: a randomized, controlled, phase II trial in Latin America. Pediatr Infect Dis J 2013, 32:1102-9). However, clinical trials have shown that Dengvaxia® can enhance, rather than reduce, the risk of severe disease due to dengue infection in individuals who had not been previously infected by a dengue virus (seronegative populations). Therefore, Dengvaxia® is only recommended for use in individuals who had been previously infected with at least one dengue virus serotype (seropositive populations). More specifically, according to the European Medicine Agency's European Public Assessment report (EPAR) for the product, Dengvaxia® is only for use in people from 9 to 45 years of age who have been infected with dengue virus before and who live in areas where this infection is endemic. Endemic areas are areas where the disease occurs regularly throughout the year. See also Sridhar S et al. Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy. N Engl J Med 2018, 379:327-40; and World Health Organization. Dengue vaccine: WHO position paper—September 2018. Wkly Epidemiol Rec 2018, 93:457-76. S. R. Hadinegoro et al. report in the New England Journal of Medicine, Vol. 373, page 1195, in "Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease" a pooled risk of hospitalization for virologically-confirmed dengue disease among those under the age of 9 years of 1.58 indicating an increased risk for the vaccinated group with respect to severe dengue. This leaves a substantial unmet need for an effective vaccine with a good safety profile in both dengue-naïve and seropositive individuals, including those dengue-naïve populations living in endemic areas, younger individuals who may not have developed any seropositive response to dengue or been exposed to dengue, and travelers and individuals from non-endemic regions. There is also a need for outbreak control or travel vaccination, offering a reduction in the risk of dengue after only one dose.

One further disadvantage of the only currently approved dengue vaccine, Dengvaxia®, is that it must only be given to people who have had a positive test result showing a previous infection with dengue virus (EPAR), i.e. individuals with known serostatus for dengue. Thus, individuals with unknown serostatus for dengue cannot be vaccinated with Dengvaxia®. There is thus a medical need for a dengue vaccine which, as well as being safe and efficacious for seropositive individuals, can also be administered to individuals with unknown dengue serostatus, children under 9 years old and seronegative individuals.

There is also a need for a vaccine that is administered in fewer doses than the current Dengvaxia® dosing schedule of 3 doses, 6 months apart, such as a vaccine that can be administered in only two doses or one dose to be efficacious.

Also, there is a need for a dengue vaccine that stimulates an immune response to all dengue serotypes, preferably a balanced immune response to all serotypes, and protects against dengue disease of any severity (including DSS, DHF), preferably both in seronegative and seropositive populations, which is safe for a larger group of ages, in particular for subjects of 9 years and younger. The development of a safe and effective vaccine capable of protecting all populations, including both seronegative and seropositive populations, and in particular children and young adults in endemic settings, represents an important approach to the prevention and control of this global disease.

OBJECTS AND SUMMARY

It is an object of the present invention to provide a safe and effective vaccine against all serotypes of dengue virus

for dengue-endemic and dengue non-endemic populations and for a broad range of ages, in particular for subjects between 2 months and 60 years of age, and independent of previous exposure to dengue virus and corresponding seropositive or seronegative status before vaccination.

It is an object of the present invention to minimize the risk of DHF and DSS caused by infection with DENV-1, DENV-2, DENV-3 or DENV-4, in particular following vaccination in children of young age and individuals of any age who have never been previously exposed to dengue, or who are seronegative to dengue before vaccination.

It is an object of the present invention to provide a vaccine which stimulates a balanced immune response to all four dengue serotypes in a subject.

The present invention is therefore directed in part to a reconstituted dengue vaccine composition for use in a method of preventing virologically confirmable dengue disease in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, wherein the dengue vaccine composition is a tetravalent dengue virus composition including four dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, optionally wherein the dengue virus strains are live, attenuated, and wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent

- (i) dengue serotype 1 has a concentration of at least $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$ and optionally to $5.0 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) dengue serotype 2 has a concentration of at least $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$ and optionally to $4.9 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) dengue serotype 3 has a concentration of at least $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$ and optionally to $5.7 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) dengue serotype 4 has a concentration of at least $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$ and optionally to $6.2 \log 10 \text{ pfu}/0.5 \text{ mL}$.

The present invention is therefore directed in part to a dengue vaccine composition for use in a method of preventing virologically confirmable dengue disease (VCD) in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, and wherein the dengue vaccine composition is a tetravalent dengue virus composition including four live, attenuated dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, wherein the attenuated dengue virus strains comprise chimeric dengue viruses and preferably at least one non-chimeric dengue virus, and wherein the dengue serotype 1 and the dengue serotype 2 are present each in a concentration based on the total concentration in pfu/0.5 mL which is within 5%-points of each other and/or are together less than about 10% of the total concentration in pfu/0.5 mL, in particular wherein the dengue serotype 3 is at least about 10% of the total concentration in pfu/0.5 mL and in particular wherein the dengue serotype 4 is at least about 70% of the total concentration in pfu/0.5 mL.

The present invention is therefore directed in part to a unit dose of a dengue vaccine composition and use thereof, the

unit dose comprising: a tetravalent dengue virus composition including four live attenuated dengue serotypes (e.g. virus strains):

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain),
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain),
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain),
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain),

and one or more pharmaceutically acceptable excipients thereof.

The present invention is further directed in part to a unit dose of a dengue vaccine composition and use thereof, the unit dose comprising:

a tetravalent virus composition including four live attenuated dengue virus strains:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of at least $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of at least $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of at least $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of at least $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$,

and one or more pharmaceutically acceptable excipients.

The present invention is further directed in part to a unit dose of a dengue vaccine composition and use thereof, the unit dose comprising:

a tetravalent virus composition including four live attenuated dengue virus strains, wherein the unit dose is lyophilized and upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent comprises:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of at least $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of at least $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of at least $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of at least $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$.

The present invention is further directed in part to a unit dose of a dengue vaccine composition and use thereof, wherein said unit dose is lyophilized and obtained by lyophilizing 0.5 mL of a solution comprising:

a tetravalent virus composition including four live attenuated dengue virus strains:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of at least $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of at least $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of at least $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of at least $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$,

and one or more pharmaceutically acceptable excipients.

The present invention is further directed in part to a kit for preparing a reconstituted unit dose and use thereof, the kit comprising the following components:

- a) a lyophilized unit dose of the present invention as described herein, and
- b) a pharmaceutically acceptable diluent for reconstitution.

The present invention is further directed in part to a container, such as a vial, comprising one to ten unit doses of the present invention as described herein.

The present invention is further directed to a method of preventing dengue disease in a subject comprising administering to the subject a reconstituted unit dose of a dengue vaccine composition as described herein, for example by subcutaneous injection.

The present invention is further directed in part to a method of preventing virologically confirmable dengue disease in a subject comprising administering to the subject a tetravalent dengue virus composition including four live, attenuated dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4, in particular without determining the serostatus of the subject at baseline, said method being safe and effective.

The present invention is further directed in part directed to such a method having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein preferably said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, and optionally at least 4 weeks apart, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule.

The present invention is further directed in part to such a method having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, and optionally at least 4 weeks apart, 30 days after the second administration until at least 12 months after the second administration.

The present invention is further directed to a method of preventing dengue disease in a subject population, comprising administering to the subject population a reconstituted unit dose of a vaccine composition as described herein, wherein the subject population is seronegative to all dengue serotypes. In said method the geometric mean neutralizing antibody titers (GMTs) when tested in at least 40, or at least 50, or at least 60 subjects at day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, may provide a ratio of not more than 50, or not more than 40, or not more than 30, or not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 4 (GMT DENV-2:GMT DENV-4), and optionally a ratio of not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 1 (GMT DENV-2:GMT DENV-1), and optionally a ratio of not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 3 (GMT DENV-2:GMT DENV-3).

The present invention is further directed to a method of preventing dengue disease in a subject, comprising admin-

istering to the subject a reconstituted unit dose of a vaccine composition as described herein, wherein the subject is seronegative to all dengue serotypes. In said method the neutralizing antibody titers when tested in the subject at day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, may provide a ratio of not more than 50, or not more than 40, or not more than 30, or not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4, and optionally a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 1, and optionally a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 3.

In one preferred embodiment, the methods of preventing dengue disease of the present invention are not associated with an increased likelihood of solicited systemic adverse events, such as in children under 9 or seronegative individuals.

The present invention is further directed in part to the use of the reconstituted unit dose of a dengue vaccine composition as described herein for the manufacture of a medicament for preventing dengue disease in a subject.

The present invention is further directed in part to the reconstituted unit dose of a dengue vaccine composition as described herein for use in a method of preventing dengue disease in a subject as described herein.

DEFINITIONS

In describing the present invention, the following terms are to be used as indicated below. As used herein, the singular forms "a," "an," and "the" include plural references unless the context clearly indicates otherwise.

As used herein, the terms "unit dose of a dengue vaccine composition", "unit dose" and "unit dose of the invention as described herein" refer to the amount of a dengue vaccine which is administered to a subject in a single dose. In one embodiment, one unit dose is present in a vial and this unit dose is administered to a subject, optionally after reconstitution. In one embodiment, more than one unit dose of the dengue vaccine composition may be present in a vial so that with the content of one vial more than one subject can be vaccinated.

A "lyophilized unit dose" or "unit dose in lyophilized form" refers to the unit dose that is obtained by subjecting a given volume of the liquid dengue vaccine composition, such as 0.5 mL, to lyophilization. Thus, the aqueous formulations of the dengue vaccine composition being produced by combining the pharmaceutically acceptable excipients and the dengue virus composition comprising the four dengue virus strains, preferably TDV-1 to TDV-4, is subjected to lyophilization to obtain the lyophilized unit dose.

A "reconstituted unit dose" or "unit dose in reconstituted form" is obtained from the lyophilized dose by reconstitution with a pharmaceutically acceptable diluent. The diluent does not contain dengue virus. The reconstituted unit dose is a liquid which can be administered to a subject, for example by injection, such as subcutaneous injection.

As used herein, the term "upon reconstitution with 0.5 mL" is not limiting the reconstitution to be performed using 0.5 mL of the diluent, but refers to the concentration of the dengue viruses that will be present in the reconstituted unit dose when 0.5 mL diluent are used for reconstitution. While using a different volume for reconstitution (e.g. 0.8 mL) will

result in a different concentration of dengue viruses in the reconstituted unit dose, the administration of the total volume of the unit dose (e.g. 0.8 mL) will result in the same total amount of dengue virus being administered.

As used herein, a “concentration of at least X log 10 pfu/0.5 mL” refers to the concentration of a dengue serotype in 0.5 mL, but is not limiting the unit dose to be 0.5 mL. If the unit dose has a volume different than 0.5 mL, or is lyophilized from a volume different than 0.5 mL, or is reconstituted with a volume different than 0.5 mL, said concentration will differ from the “concentration of at least X log 10 pfu/0.5 mL”. However, if the unit dose has a volume of 0.5 mL, or is lyophilized from a volume of 0.5 mL, or is reconstituted with a volume of 0.5 mL, said concentration will be the “concentration of at least X log 10 pfu/0.5 mL”. Thus, while the concentration may differ, the total amount of virus in the unit dose remains the same.

As used herein, the term “dengue serotype” refers to a species of dengue virus which is defined by its cell surface antigens and therefore can be distinguished by serological methods known in the art. At present, four serotypes of dengue virus are known, i.e. dengue serotype 1 (DENV-1), dengue serotype 2 (DENV-2), dengue serotype 3 (DENV-3) and dengue serotype 4 (DENV-4).

As used herein, the term “tetravalent dengue virus composition” refers to a dengue virus composition comprising four different immunogenic components from the four different dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4, preferably comprising four different live, attenuated dengue viruses, each representing one dengue serotype, and which aims to stimulate immune responses to all four dengue serotypes.

As used herein, the term “live attenuated dengue virus” refers to a viable dengue virus which is mutated to provide reduced virulence. The live attenuated dengue virus can be a dengue virus in which all components are derived from the same dengue serotype or it can be a chimeric dengue virus having parts from two or more dengue serotypes.

A “virus strain” and in particular a “dengue virus strain” is a genetic subtype of a virus, in particular of a dengue virus, which is characterized by a specific nucleic acid sequence. A dengue serotype may comprise different strains with different nucleic acid sequences which have the same cell surface antigens. A dengue virus strain can be a dengue virus in which all components are derived from the same dengue serotype or it can be a chimeric dengue virus having parts from two or more dengue serotypes.

As used herein, “TDV-2” refers to a molecularly characterized and cloned dengue serotype 2 strain derived from the live attenuated DEN-2 PDK-53 virus strain. The PDK-53 strain is described for example in Bhamaraprabhati et al. (1987) Bulletin of the World Health Organization 65(2): 189-195. In one embodiment, the TDV-2 strain served as a backbone for the chimeric TDV-1, TDV-3 and TDV-4 strains into which parts from the TDV-1, TDV-3 and TDV-4 strains were introduced.

A “non-chimeric dengue virus” or “non-chimeric dengue serotype strain” or “non-chimeric dengue strain” comprises only parts from one dengue serotype. In particular, a non-chimeric dengue virus does not include parts from a different flavivirus such as yellow fever virus, Zika virus, West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, tick-borne encephalitis virus. TDV-2 is an example of a non-chimeric dengue virus.

A “chimeric dengue virus” or “chimeric dengue serotype strain” or “chimeric dengue strain” comprises parts from at least two different dengue serotypes. As used herein, the

chimeric dengue virus does not include parts from a different flavivirus such as yellow fever virus, Zika virus, West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, tick-borne encephalitis virus. In particular, the chimeric dengue virus described herein does not include parts from the yellow fever virus. As used herein, a “chimeric dengue serotype 2/1 strain” or “DENV-2/1 chimera” or “TDV-1” refers to a dengue virus chimeric construct which comprises parts from both DENV-2 and DENV-1. In particular, in the chimeric dengue serotype 2/1 strain the prM and E proteins from DENV-1 replace the prM and E proteins from DENV-2 as detailed below. As used herein, a “chimeric dengue serotype 2/3 strain” or “DENV-2/3 chimera” or “TDV-3” refers to a dengue virus chimeric construct which comprises parts from both DENV-2 and DENV-3. In particular, in the chimeric dengue serotype 2/3 strain the prM and E proteins from DENV-3 replace the prM and E proteins from DENV-2 as detailed below. As used herein, a “chimeric dengue serotype 2/4 strain” or “DENV-2/4 chimera” or “TDV-4” refers to a dengue virus chimeric construct which comprises parts from both DENV-2 and DENV-4. In particular, in the chimeric dengue serotype 2/4 strain the prM and E proteins from DENV-4 replace the prM and E proteins from DENV-2 as detailed below.

As used herein, “TDV” refers to a tetravalent live attenuated dengue vaccine that comprises a mixture of the four live attenuated dengue virus strains TDV-1, TDV-2, TDV-3 and TDV-4 expressing surface antigens from the four dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4, respectively. In one embodiment, TDV-1 has the nucleotide sequence according to SEQ ID No. 1 and/or the amino acid sequence according to SEQ ID No. 2. In one embodiment, TDV-2 has the nucleotide sequence according to SEQ ID No. 3 and/or the amino acid sequence according to SEQ ID No. 4. In one embodiment, TDV-3 has the nucleotide sequence according to SEQ ID No. 5 and/or the amino acid sequence according to SEQ ID No. 6. In one embodiment, TDV-4 has the nucleotide sequence according to SEQ ID No. 7 and/or the amino acid sequence according to SEQ ID No. 8.

As used herein, the term “dengue disease” refers to the disease which is caused by infection with dengue virus. Symptoms of dengue disease include sudden high fever, headaches, joint and muscle pain, nausea, vomiting and skin rashes. The term dengue disease also includes the more severe forms of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Symptoms of DHF include increased vascular permeability, hypovolemia and abnormal blood clotting mechanisms. Subjects with DHF may present with severe manifestations of plasma leakage and hemorrhage. When a subject with DHF experiences shock he or she will be categorized as having DSS. Symptoms of DSS include bleeding that may appear as tiny spots of blood on the skin and larger patches of blood under the skin. Prolonged shock is the main factor associated with complications including massive gastrointestinal hemorrhage that can lead to death. As used herein, DHF cases are defined as VCD cases meeting WHO 1997 DHF criteria.

As used herein, “preventing dengue disease” refers to preventing a subject from developing one or more symptoms of dengue disease because of an infection with a dengue virus. In particular, preventing dengue disease is achieved by vaccinating or inoculating a subject with a dengue vaccine composition, such as the reconstituted unit dose described herein. As used herein, the term “prophylactically treating dengue disease” is equivalent to “preventing dengue dis-

ease". In a particular embodiment, preventing dengue disease includes preventing DHS and/or DSS.

As used herein, the terms "virologically-confirmed dengue disease", "VCD case", or "VCD fever" refer to febrile illness or illness clinically suspected to be dengue disease with a positive serotype-specific reverse transcriptase polymerase chain reaction (RT-PCR). The term "virologically confirmable dengue" disease refers to a subject having febrile illness or illness clinically suspected to be dengue disease, wherein testing the subject, e.g. using RT-PCR, would confirm the presence of at least one dengue serotype. Severe forms of VCD fever will be identified as follows: Dengue Hemorrhagic Fever (DHF) was defined according to the WHO 1997 criteria. Severe dengue was defined through an assessment of an independent Dengue Case Adjudication Committee which will assess all hospitalized VCD cases (severe/non-severe) based on criteria redefined in a charter. All non-hospitalized cases are considered non-severe.

As used herein, the term "febrile illness" is defined as temperature $\geq 38^\circ\text{C}$. on any 2 of 3 consecutive days.

As used herein, the terms "virologically-confirmed dengue disease with hospitalization", is considered to be a surrogate for severe dengue and the "incidence of virologically-confirmed dengue disease with hospitalization" is used as a safety parameter. As used herein, the "relative risk with respect to virologically-confirmed dengue disease with hospitalization" means the number of events of virologically confirmed dengue disease with hospitalization divided by the number of subjects treated with the unit dose as disclosed herein over the number of events of virologically confirmed dengue disease with hospitalization divided by the number of subjects treated with placebo. If the "relative risk with respect to virologically-confirmed dengue disease with hospitalization" is 1 or lower the vaccine provides for the same or less risk for virologically-confirmed dengue disease with hospitalization as placebo and is considered "safe". In this context the risk of virologically-confirmed dengue disease with hospitalization may be also 0.9 or less, 0.8 or less, 0.7 or less, 0.6 or less, 0.5 or less, 0.4 or less, 0.3 or less, 0.2 or less, or 0.1 or less, in particular when determined from 30 days after a second administration until 12 months after a second administration, in particular when determined in age groups selected from the age group of 4 to 16 year old subjects, the age group of 4 to under 9 year old subjects, the age group of 2 to under 9 year old subjects, the age group of 4 to 5 year old subjects, the age group of 6 to 11 year old subjects, and the age group of 12 to 16 year old subjects.

As used herein, alternatively a vaccine is considered "safe" when the vaccine efficacy (VE) with respect to virologically-confirmed dengue disease with hospitalization is 0% or higher. This means that the vaccine provides for the same likelihood or less for virologically-confirmed dengue disease with hospitalization as placebo. In particular considered "safe" is the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, in particular when measured against placebo in a subject population of at least 2,000 healthy subjects (in particular when measured in age groups selected from the age group of 4 to 16 year old subjects, the age group of 4 to under 9 year old subjects, the age group of 2 to under 9 year old subjects, the age group of 4 to 5 year old subjects, the age group of 6 to 11 year old subjects, and the age group of 12 to 16 year old subjects) being seronegative against all serotypes at baseline or being seropositive against at least one serotype at baseline, in particular when said unit dose or said placebo is adminis-

tered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In particular, the lower bound may be more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, or more than 75%. In particular, the 2-sided 95% confidence interval of the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes when comparing seropositive and seronegative subjects provides for lower bounds of the 2-sided confidence interval which are within 10% points.

If one of the criteria as defined above for the term "safe" is fulfilled, the vaccine is considered safe within the meaning of this invention. In this context, safe in particular refers to a vaccine that is safe for all subjects irrespective of their serostatus at baseline. This means that the vaccine can be administered without the need to determine the occurrence of a previous dengue infection in the subject before administration. Preferably, the vaccine is safe as defined above with respect to all age groups starting from 4 years of age and preferably irrespective of the serostatus, in particular from 4 years of age to 60 years of age, or 4 years of age to 16 years of age. Relevant subgroups in this context are under 9 years of age, from 2 years of age to under 9 years of age, from 4 years of age to under 9 years of age, 4 to 5 years of age, 6 to 11 years of age and 12 to 16 years of age or any age group within 4 to 16 years of age.

As used herein, "vaccine efficacy" or "VE" measure the proportionate reduction in cases among vaccinated persons. Vaccine efficacy (VE) is measured by calculating the risk of disease among vaccinated and unvaccinated persons and determining the percentage reduction in risk of disease among vaccinated persons relative to unvaccinated persons. The greater the percentage reduction of illness in the vaccinated group, the greater the vaccine efficacy. For example, a VE of 90% indicates a 90% reduction in disease occurrence among the vaccinated group, or a 90% reduction from the number of cases you would expect if they have not been vaccinated. The vaccine efficiency is calculated by the formula: $100*(1-\text{HR})$, wherein HR is the Hazard Ratio which is defined as the Hazard rate of vaccine (λ_v) divided by the Hazard rate of placebo (λ_c), i.e. $\text{HR} = \lambda_v / \lambda_c$. λ_v denote the hazard rate for the subjects vaccinated with a tetravalent dengue vaccine composition as disclosed herein and λ_c denote the hazard rate for unvaccinated subjects, i.e. subjects receiving placebo. The hazard rate ratio HR is estimated from a Cox proportional hazard model with study vaccine as a factor, adjusted for age, and stratified by region. As used herein the term "combined vaccine efficacy against all four serotypes" is defined as the vaccine efficacy in relation to the risk of dengue disease irrespective of the serotype being responsible for the virologically-confirmed dengue disease and the subject baseline serostatus. A vaccine is considered "effective" in case the combined vaccine efficacy is above 30%. In this context the combined vaccine efficacy may be also 40% or more, 50% or more, 60% or more, 70% or more, or 80% or more, in particular when determined from 30 days after a second administration until 12 months after a second administration, in particular when determined in age groups selected from the age group of 4 to 16 year old subjects, the age group of 4 to under 9 year old subjects, the age group of 2 to under 9 year old subjects, the age group of 4 to 5 year old subjects, the age group of 6 to 11 year old subjects, and the age group of 12 to 16 year old subjects. In this context, effective in particular refers to a vaccine that is effective for all subjects irrespective of their serostatus at baseline. Pref-

erably, the vaccine is effective with respect to all age groups starting from 4 years of age and preferably irrespective of the serostatus, in particular from 4 years of age to 60 years of age or from 4 years of age to 16 years of age and irrespective of the serostatus. Relevant subgroups in this context are under 9 years of age, from 2 years of age to under 9 years of age, from 4 years of age to under 9 years of age, 4 to 5 years of age, 6 to 11 years of age and 12 to 16 years of age or any age group within 4 to 16 years of age. Further specific efficacies can be defined. As used herein, "combined vaccine efficacy against all four serotypes in seronegative subjects" refers to the efficacy measured in subjects which are seronegative at baseline. As used herein, "vaccine efficacy against a specific serotype, e.g. serotype 1" refers to the efficacy in relation to a specific serotype being responsible for the virologically-confirmed dengue disease. As used herein, "combined vaccine efficacy against all four serotypes against virologically-confirmed dengue with hospitalization" refers to the efficacy wherein only virologically-confirmed dengue cases with hospitalization are considered. Such vaccine efficacies can be determined with respect to subjects being seronegative or seropositive at baseline and for different age groups.

As used herein, the "relative risk" means the number of events of virologically confirmed dengue disease divided by the number of subjects treated with the unit dose as disclosed herein over the number of events of virologically confirmed dengue disease divided by the number of subjects treated with placebo. As used herein the term "combined relative risk against all four serotypes" is defined as the relative risk in relation to the risk of dengue disease irrespective of the serotype being responsible for the virologically-confirmed dengue disease and the subject baseline serostatus.

As used herein, "vaccinating" or "inoculating" refers to the administration of a vaccine to a subject with the aim to prevent the subject from developing one or more symptoms of a disease. As used herein, "vaccinating against dengue disease" or "inoculating against dengue disease" refers to the administration of a dengue vaccine composition to a subject with the aim to prevent the subject from developing one or more symptoms of dengue disease.

As used herein, the terms "subject" or "subjects" are limited to human subjects (e.g. infants, children or adults).

As used herein, "subject population" refers to a group of subjects. The subject population may refer to least 40 subjects, at least 50 subjects, at least 60 subjects, at least 100 subjects or at least 1000 subjects and is defined by certain parameters. The parameters that may be used to define a subject population include, but are not limited to, the age of the subjects, whether the subjects are from a dengue endemic region or from a dengue non-endemic region and the serostatus of the subjects.

As used herein, "endemic region" refers to a region where a disease or infectious agent is constantly present and/or usually prevalent in a population within this region. As used herein, "non-endemic region" refers to a region from which the disease is absent or in which it is usually not prevalent. Accordingly, a "dengue endemic region" refers to geographic areas in which an infection with dengue virus is constantly maintained at a baseline level. A "dengue non-endemic region" is a geographic area in which an infection with dengue virus is not constantly maintained at a baseline level. Accordingly, subject populations or subjects "from a dengue endemic region" or "from a dengue non-endemic region" refer to subject populations or subjects living in geographic areas as defined above. Whether a geographic area or a subject population is dengue-endemic or not can be

determined by different calculatory methods such as the ones described in Bhatt et al. (2013) *Nature* 496 (7446): 504-507 and supplementary material and in Stanaway et al. (2016) *Lancet Infect Dis.* 16(6): 712-723 and supplementary material. Overviews of dengue endemic regions and dengue epidemiology are regularly published, for example, by the WHO or CDC. Typical dengue-endemic regions are in Latin America, Southeast Asia and the Pacific islands and dengue endemic countries include, but are not limited to, Australia, Brazil, Bangladesh, Colombia, China, Dominican Republic, Indonesia, India, Mexico, Malaysia, Nicaragua, Nigeria, Pakistan, Panama, Philippines, Puerto Rico, Singapore, Sri Lanka, Thailand and Vietnam.

As used herein, "serostatus" refers to the amount of antibodies a subject has with respect to a certain infectious agent, in particular dengue virus. As used herein, "seronegative" or "seronaïve" means that the subject does not have neutralizing antibodies against any one of dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4 in the serum. A seronegative or seronaïve subject or subject population is defined by a neutralizing antibody titer of less than 10 for each one of the four dengue serotypes. A subject or subject population having a neutralizing antibody titer of equal to or more than 10 for at least one dengue serotype is defined as being "seropositive" with respect to said dengue serotype. Serostatus at baseline refers to the serostatus before the administration of a dengue vaccine composition as described herein.

As used herein, a "neutralizing antibody titer" refers to the amount of antibodies in the serum of a subject that neutralize the respective dengue serotype. The neutralizing antibody titer against DENV-1, DENV-2, DENV-3 and DENV-4 is determined in a serum sample of the subject using known methods such as the plaque reduction neutralization test (PRNT) as described in the WHO Guidelines (World Health Organization Department of Immunization Vaccines Biologicals (2007) Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses, WHO/IVB/07.07) or a microneutralization (MNT50) assay as described herein. As used herein, the "ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4" means that the neutralizing antibody titer of dengue serotype 2 is divided by the neutralizing antibody titer of dengue serotype 4 and that the ratio obtained hereby is no more than 20. In other words, the neutralizing antibody titer of dengue serotype 2 is not more than 20-times higher than the neutralizing antibody titer of dengue serotype 4 in the subject.

As used herein, the terms "geometric mean neutralizing antibody titer" and "GMT" refer to the geometric mean value of the titer of neutralizing antibodies against the corresponding dengue serotype in the serum of subjects in a subject population. The geometric mean value is calculated by a well-known formula. As used herein, the "ratio of not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 4" means that the geometric mean neutralizing antibody titer of dengue serotype 2 (GMT DENV-2) is divided by the geometric mean neutralizing antibody titer of dengue serotype 4 (GMT DENV-4) and that the ratio obtained hereby is no more than 20. In other words, the geometric mean neutralizing antibody titer of dengue serotype 2 is not more than 20-times higher than the geometric mean neutralizing antibody titer of dengue serotype 4 in the subject population.

As used herein, an "immune response" refers to a subjects response to the administration of the dengue vaccine. In particular, the immune response includes the formation of

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neutralizing antibodies to one or more dengue serotypes. It may also include the stimulation of a cell-mediated response or the formation of antibodies to non-structural proteins such as NS1. An immune response is stimulated by the administration of a unit dose of the invention as described herein, if the titer of neutralizing antibodies against at least one dengue virus serotype and preferably against all four dengue virus serotypes is increased after said administration of said unit dose. An immune response is stimulated by the administration of a unit dose of the invention as described herein, if the secretion of interferon gamma by peripheral blood mononuclear cells stimulated with peptides from dengue virus proteins is increased after said administration of said unit dose. An immune response is stimulated by the administration of a unit dose of the invention as described herein, if the titer of antibodies to non-structural proteins such as NS1 is increased after said administration of said unit dose. In a particular embodiment, the administration of a reconstituted unit dose of the present invention as described herein stimulates the formation of neutralizing antibodies to one or more dengue serotypes, a cell-mediated response and the formation of antibodies to non-structural proteins such as NS1.

As used herein, a “balanced immune response” means that the immune response to the four dengue serotypes is sufficient to provide protection against infection by all four dengue serotypes and preferably the immune response to the four dengue serotypes has a similar strength. In particular, the neutralizing antibody titer against the four dengue serotypes at day 180 or day 365 after administration of a first reconstituted unit dose of the invention as described herein is similar, i.e. it differs by less than factor 30, by less than factor 25 or by less than factor 20.

As used herein, “solicited systemic adverse events” in children under 6 years are defined as fever, irritability/fussiness, drowsiness and loss of appetite that occurred within 14 days after each vaccination, and in children of 6 years or more are defined as fever, headache, asthenia, malaise and myalgia that occurred within 14 days after each vaccination.

As used herein, “solicited local adverse events” are injection site pain, injection site erythema and injection site swelling that occurred within 7 days after each vaccination.

As used herein, “unsolicited adverse events” are any adverse events (AEs) that are not solicited local or systemic AEs, as defined above.

As used herein, a “serious adverse event” or “SAE” is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect or is medically important due to other reasons than the above mentioned criteria.

The relationship of each AE, including solicited systemic AEs (solicited local AEs are considered as related) to trial vaccine(s) will be assessed using the following categories: As used herein, “IP-Related AE” or “vaccine related AE” means that there is suspicion that there is a relationship between the vaccine and the AE (without determining the extent of probability); there is a reasonable possibility that the vaccine contributed to the AE. As used herein, “Non-IP Related” or “non-vaccine related” means that there is no suspicion that there is a relationship between the vaccine and the AE; there are other more likely causes and administration of the vaccine is not suspected to have contributed to the AE.

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As used herein, a subject or subject population being “2 to 17 years of age” refers to a subject or subject population being 2 to 17 years of age on the first day of the administration of the dengue vaccine composition as described herein.

As used herein “%-points” refers to the difference of two %-values in a %-value. For example two values in % which are within 5%-points refers to e.g. one value at 1% and a second value at 6%.

As used herein, the term “determination of the previous dengue infection in the subject before administration” means that a previous dengue infection has to be assessed before vaccination in that there is a laboratory confirmed history of dengue or through an appropriately validated serological test e.g. by the method as disclosed herein such as the MNT50 test described in Example 2 or any serotesting with adequate performance in terms of specificity and cross reactivity based on the locale disease epidemiology.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Genetic structure of the four dengue strains contained in TDV. The solid red triangles indicate the three attenuating mutations present in the 5'NCR, NS1 and NS3 proteins. The TDV-1, TDV-3 and TDV-4 strains are chimeric viruses where the prM and E genes from dengue serotype 1, 3 and 4, respectively, are inserted into the TDV-2 backbone.

FIG. 2: Schematic drawing illustrating the microneutralization test (MNT) used to determine the titer of neutralizing antibodies.

FIGS. 3A & 3B: Percentage of subjects ($\pm 95\%$ confidence intervals) who were seropositive (reciprocal neutralizing titer ≥ 10) for each of the dengue serotypes at different time points of the trial in the HD-TDV group (dark colored, left bar) and TDV group (light colored, right bar) throughout the trial. Time points shown are baseline, day 15 (d15), day 30 (d30), day 90 (d90), day 180 (d180) and day 365 (d365). FIG. 3A shows the results for participants seropositive (set of graphs on the left) and seronegative (set of graphs on the right) at baseline, per-protocol set. FIG. 3B shows the results for the entire trial population (all) per-protocol set

FIGS. 4A & 4B: Geometric mean titers (GMTs) ($\pm 95\%$ confidence intervals) of neutralizing antibodies against each of the four dengue serotypes during the course of the trial for HD-TDV (dark line with triangles) and TDV (light line with circles) recipients, for the entire trial population (FIG. 4B) and for participants seropositive and seronegative at baseline (FIG. 4A), per-protocol set.

FIG. 5: IFNy ELISpot analysis of peripheral blood mononuclear cells before vaccination (baseline) and at different time points after administration of TDV. Shown are the response frequencies to the entire DENV-2 proteome. A subject was considered responsive if response to more than one peptide pool from DENV-2 was positive (i.e. $\geq 4\times$ mean of negative control and ≥ 50 SFC/ 10^6 PBMCs).

FIGS. 6A & 6B: IFNy ELISpot analysis of peripheral blood mononuclear cells before vaccination (baseline) and at different time points after administration of TDV. Shown are the response frequencies to peptide pools matching selected DENV-derived proteins as indicated. A subject was considered responsive if response to more than one peptide pool from DENV-2 was positive (i.e. $\geq 4\times$ mean of negative control and ≥ 50 SFC/ 10^6 PBMCs). A=DENV-2 C; B=DENV-1 prM+E; C=DENV-2 prM+E; D=DENV-3 prM+E; E=DENV-4 prM+E; F=DENV-2 NS1; G=DENV-2 NS2; H=DENV-2 NS3; I=DENV-2 NS4; J=DENV-2 NS5.

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FIGS. 7A & 7B: Effect of sera from a seronegative subject (FIG. 7A) and a seropositive subject (FIG. 7B) to whom TDV was administered on DENV-2 NS1-induced hyperpermeability as determined by TEER. HPMEC were grown on Transwell semi-permeable membranes (0.4 µm pore size), and serum samples (30 µl) were added to the apical chamber in the presence or absence of DENV2 NS1 (5 µg/ml). DENV2 NS1 is depicted as squares; day 0 serum alone is depicted as diamonds; day 120 serum alone is depicted as triangles; day 0 serum+DENV2 NS1, is depicted as upside-down triangles; day 120 serum+DENV2 NS1 is depicted as X's. (.) represents media change. Endothelial permeability was measured at indicated time-points over 48 hours. Relative TEER values from one independent experiment performed in duplicate are plotted. Error bars indicate standard error of the mean (SEM).

FIGS. 8A & 8B: Effect of sera from seronegative and seropositive subjects to which TDV was administered on NS1-induced sialic acid and heparan sulfate degradation. Shown is the quantification of mean fluorescence intensity (MFI) of (FIG. 8A) sialic acid and (FIG. 8B) heparan sulfate expression after staining with sialic acid- and heparan sulfate-specific fluorescent antibodies as visualized by confocal microscopy. Values are normalized to MFI from the NS1+ positive control serum group (represented by dotted line at 100%) and expressed as percentage of control. Error bars indicate SEM. The left bar for each subject shows the results at day 0 (d0), the right bar for each subject shows the results at day 120 (d120).

FIG. 9: Flow diagram of the clinical trial of Example 6.

FIGS. 10A & 10B: Cumulative incidence of FIG. 10A) virologically-confirmed dengue cases and FIG. 10B) hospitalized virologically-confirmed dengue cases over time during Part 1 study period by baseline serostatus (safety set data; data presented truncated at Month 18). Tables show numbers of participants under follow-up at various time points to end of Part 1 study period.

DETAILED DESCRIPTION

Dengue Virus Strains

The dengue virus is a single stranded, positive sense RNA virus of the family flaviviridae. The taxonomy is outlined in Table 1. The family flaviviridae includes three genera, flavivirus, hepacivirus and pestivirus. The genus flavivirus contains highly pathogenic and potentially hemorrhagic fever viruses, such as yellow fever virus and dengue virus, encephalitic viruses, such as Japanese encephalitis virus, Murray Valley encephalitis virus and West Nile virus, and a number of less pathogenic viruses.

TABLE 1

Dengue Virus Taxonomy of the GMO Parental Strain	
Family	Flaviviridae
Genus	Flavivirus
Species	Dengue virus
Strains	Dengue Serotype 2 (Strain 16681), Strain DEN-2 PDK-53
GMO parent	TDV-2

The flavivirus genome comprises in 5' to 3' direction (see FIG. 1):

- a 5'-noncoding region (5'-NCR),
- a capsid protein (C) encoding region,
- a pre-membrane protein (prM) encoding region,
- an envelope protein (E) encoding region,

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a region encoding nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) and a 3' noncoding region (3'-NCR).

The viral structural proteins are C, prM and E, and the nonstructural proteins are NS1 to NS5. The structural and nonstructural proteins are translated as a single polyprotein and processed by cellular and viral proteases.

The unit dose of the invention as described herein comprises a dengue virus composition that comprises four live attenuated dengue virus strains (tetravalent dengue virus composition) representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4. Preferably the composition comprises chimeric dengue viruses and optionally at least one non-chimeric dengue virus, in particular a molecularly characterized and cloned dengue serotype 2 strain derived from the live attenuated DEN-2 PDK-53 virus strain (TDV-2), and three chimeric dengue strains derived from the TDV-2 strain by replacing the structural proteins prM and E from TDV-2 with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera (TDV-1),
- a DENV-2/3 chimera (TDV-3) and
- a DENV-2/4 chimera (TDV-4).

The genetically modified tetravalent dengue vaccine TDV is based on a molecularly characterized and cloned dengue-2 virus strain (TDV-2). This attenuated TDV-2 strain was generated by cDNA cloning of the attenuated laboratory-derived DEN-2 PDK-53 virus strain that was originally isolated at Mahidol University, Bangkok, Thailand (Kinney et al. (1997) *Virology* 230(2): 300-308). DEN-2 PDK-53 was generated by 53 serial passages in primary dog kidney (PDK) cells at 32° C. (Bhamarapravati et al. (1987) *Bull. World Health Organ.* 65(2): 189-195).

The attenuated DEN-2 PDK-53 strain (the precursor of TDV-2) was derived from the wild type virus strain DEN-2 16681 and differs in nine nucleotides from the wild type as follows (Kinney et al. (1997) *Virology* 230(2): 300-308):

- (i) 5'-noncoding region (NCR)-57 (nt-57 C-to-T): major attenuation locus
- (ii) prM-29 Asp-to-Val (nt-524 A-to-T)
- (iii) nt-2055 C-to-T (E gene) silent mutation
- (iv) NS1-53 Gly-to-Asp (nt-2579 G-to-A): major attenuation locus
- (v) NS2A-181 Leu-to-Phe (nt-4018 C-to-T)
- (vi) NS3-250 Glu-to-Val (nt-5270 A-to-T): major attenuation locus
- (vii) nt-5547 (NS3 gene) T-to-C silent mutation
- (viii) NS4A-75 Gly-to-Ala (nt-6599 G-to-C)

* nt-8571 C-to-T (NS5 gene) silent mutation

The three nucleotide changes located in the 5' noncoding region (NCR) (nucleotide 57) (mutation (i)), the NS-1 (amino acid 828 of SEQ ID NO. 4) (mutation (iv)) and NS-3 genes (amino acid 1725 of SEQ ID NO. 4) (mutation (vi)) form the basis for the attenuation phenotype of the DEN-2 PDK-53 strain (Butrapet et al. (2000) *J. Virol.* 74(7): 3111-3119) (Table 2). These three mutations are referred to herein as the "attenuating mutations" and are comprised in TDV-1, TDV-2, TDV-3 and TDV-4.

TABLE 2

Attenuating mutations in the common genetic backbone of all TDV strains		
Location of Mutation	Nucleotide Change in TDV-2	Amino Acid Change in TDV-2
5' Noncoding Region (5'NCR)	57 C to T	Not applicable (silent)
Nonstructural Protein 1 (NS1)	2579 G to A	828 Gly to Asp
Nonstructural Protein 3 (NS3)	5270 A to T	1725 Glu to Val

In one embodiment, TDV-2 comprises in addition to the three attenuating mutations one or more mutations selected from:

- a) a mutation in the prM gene at nucleotide 524 from adenine to thymidine resulting in an amino acid change at position 143 from asparagine to valine, and/or
- b) a silent mutation in the E gene at nucleotide 2055 from cytosine to thymidine, and/or
- c) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymidine resulting in an amino acid change at position 1308 from leucine to phenylalanine, and/or
- d) a silent mutation in the NS3 gene at nucleotide 5547 from thymidine to cytosine, and/or
- e) a mutation in the NS4A gene at nucleotide 6599 from guanine to cytosine resulting in an amino acid change at position 2168 from glycine to alanine, and/or
- f) a silent mutation in the prM gene at nucleotide 900 from thymidine to cytosine.

The silent mutation in the NS5 gene at nucleotide 8571 from cytosine to thymidine of DEN-2 PDK-53 is not present in the TDV-2 strain.

In another embodiment, TDV-2 comprises in addition to the three attenuating mutations one or more mutations selected from:

- g) a mutation in the prM gene at nucleotide 592 from adenine to guanine resulting in an amino acid change at position 166 from lysine to glutamine, and/or
- h) a mutation in the NS5 gene at nucleotide 8803 from adenine to guanine resulting in an amino acid change at position 2903 from isoleucine to valine.

In another embodiment, TDV-2 comprises in addition to the three attenuating mutations the mutations a) and g), preferably the mutations a), g), c), e) and h), more preferably the mutations a), g), c), e), h) and b), even more preferably the mutations a), g), c), e), h), b) and d), and most preferably the mutations a) to h). The nucleotide positions and amino acids positions of TDV-2 refer to the nucleotide sequence as shown in SEQ ID NO. 3 and amino acid sequence as shown in SEQ ID NO. 4.

The dengue virus structural envelope (E) protein and pre-membrane (prM) protein have been identified as the primary antigens that elicit a neutralizing protective antibody response (Plotkin 2001). For creation of the tetravalent dengue vaccine (TDV), TDV-2 was modified by replacing the nucleic acid sequence encoding the DENV-2 prM and E glycoproteins with the nucleic acid sequence encoding the corresponding wild type prM and E glycoproteins from the DENV-1, DENV-3, and DENV-4 wild type strains DENV-1 16007, DENV-3 16562 or DENV-4 1036 virus, respectively, (see Table 3) using standard molecular genetic engineering methods (Huang et al. (2003) J. Virol. 77(21): 11436-11447).

TABLE 3

Viral origin of prM/E gene regions of the TDV virus strains				
Virus	Strain	Origin	Source	Reference
DENV-1	16007	Thailand, 1964	DHF/DSS patient	Halstead and Simasthiem, 1970
DENV-2	16681	Thailand, 1964	DHF/DSS patient	Halstead and Simasthiem, 1970
DENV-3	16562	Philippines, 1964	DHF patient	Halstead and Simasthiem, 1970
DENV-4	1036	Indonesia, 1976	DF patient	Gubler et al., 1979

A diagram of the four TDV strains comprised in the dengue vaccine composition is shown in FIG. 1.

The chimeric dengue strains TDV-1, TDV-3 and TDV-4 express the surface antigens prM and E of the DENV-1, DENV-3 or DENV-4 viruses, as depicted in Table 3 respectively, and retain the genetic alterations responsible for the attenuation of TDV-2. Thus, each of the TDV-1, TDV-3 and TDV-4 strains comprises the attenuating mutations described in Table 2.

In one embodiment, TDV-1 comprises in addition to the three attenuating mutations one or more mutations selected from:

- c) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymidine resulting in an amino acid change at position 1308 from leucine to phenylalanine, and/or
- d) a silent mutation in the NS3 gene at nucleotide 5547 from thymidine to cytosine, and/or
- e) a mutation in the NS4A gene at nucleotide 6599 from guanine to cytosine resulting in an amino acid change at position 2168 from glycine to alanine, and/or
- i) a silent mutation in the E gene at nucleotide 1575 from thymidine to cytosine, and/or
- j) a silent mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotide 453 from adenine to guanine, and/or
- k) a mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotides 2381/2382 from thymidine-guanine to cytosine-cytosine resulting in an amino acid change at position 762 from valine to alanine.

In another embodiment, TDV-1 comprises in addition to the three attenuating mutations one or more mutations selected from:

- l) a mutation in the NS2A gene at nucleotide 3823 from adenine to cytosine resulting in an amino acid change at position 1243 from isoleucine to leucine, and/or
- m) a mutation in the NS2B gene at nucleotide 4407 from adenine to thymidine resulting in an amino acid change at position 1437 from glutamine to asparagine, and/or
- n) a silent mutation in the NS4B gene at nucleotide 7311 from adenine to guanine.

In another embodiment, the TDV-1 strain comprises in addition to the three attenuating mutations the mutations l) and m), preferably the mutations l), m), c) and e), even more preferably the mutations l), m), c), e), d) and n), and most preferably the mutations l), m), c), e), d), n), i), j) and k). The nucleotide positions and amino acids positions of TDV-1 refer to the nucleotide sequence as shown in SEQ ID NO. 1 and amino acid sequence as shown in SEQ ID NO. 2.

In one embodiment, TDV-3 comprises in addition to the three attenuating mutations one or more mutations selected from:

- c) a mutation in the NS2A gene at nucleotide 4012 from cytosine to thymidine resulting in an amino acid change at position 1306 from leucine to phenylalanine, and/or

- d) a silent mutation in the NS3 gene at nucleotide 5541 from thymidine to cytosine, and/or
- e) a mutation in the NS4A gene at nucleotide 6593 from guanine to cytosine resulting in an amino acid change at position 2166 from glycine to alanine, and/or
- j) a silent mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotide 453 from adenine to guanine, and/or
- k) a mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotides 2375/ 2376 from thymidine-guanine to cytosine-cytosine resulting in an amino acid change at position 760 from valine to alanine, and/or
- o) a silent mutation in the prM gene at nucleotide 552 from cytosine to thymidine, and/or
- p) a mutation in the E gene at nucleotide 1970 from adenine to thymidine resulting in an amino acid change at position 625 from histidine to leucine.

In another embodiment, TDV-3 comprises in addition to the three attenuating mutations one or more mutations selected from:

- q) a mutation in the E gene at nucleotide 1603 from adenine to thymidine resulting in an amino acid change at position 503 from threonine to serine, and/or
- r) a silent mutation in the NS5 gene at nucleotide 7620 from adenine to guanine.

In another embodiments, TDV-3 comprises in addition to the three attenuating mutations the mutations p) and q), preferably the mutations p), q), c) and e), even more preferably the mutations p), q), c), e), d) and r), and most preferably the mutations p), q), c), e), d), r), j), k) and o). The nucleotide positions and amino acids positions of TDV-3 refer to the nucleotide sequence as shown in SEQ ID NO. 5 and amino acid sequence as shown in SEQ ID NO. 6.

In one embodiment, TDV-4 comprises in addition to the three attenuating mutations one or more mutations selected from:

- c) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymidine resulting in an amino acid change at position 1308 from leucine to phenylalanine, and/or
- d) a silent mutation in the NS3 gene at nucleotide 5547 from thymidine to cytosine, and/or
- e) a mutation in the NS4A gene at nucleotide 6599 from guanine to cytosine resulting in an amino acid change at position 2168 from glycine to alanine, and/or
- j) a silent mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotide 453 from adenine to guanine, and/or
- k) a mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotides 2381/ 2382 from thymidine-guanine to cytosine-cytosine resulting in an amino acid change at position 762 from valine to alanine, and/or
- s) a mutation in the C gene at nucleotide 396 from adenine to cytosine resulting in an amino acid change at position 100 from arginine to serine, and/or
- t) a silent mutation in the E gene at nucleotide 1401 from adenine to guanine, and/or
- u) a mutation in the E gene at nucleotide 2027 from cytosine to thymidine resulting in an amino acid change at position 644 from alanine to valine, and/or
- v) a mutation in the E gene at nucleotide 2275 from adenine to cytosine resulting in an amino acid change at position 727 from methionine to leucine.

In another embodiment, TDV-4 comprises in addition to the three attenuating mutations one or more mutations selected from:

- w) a silent mutation in the C gene at nucleotide 225 from adenine to thymidine, and/or
- x) a mutation in the NS2A gene at nucleotide 3674 from adenine to guanine resulting in an amino acid change at position 1193 from asparagine to glycine, and/or
- y) a mutation in the NS2A gene at nucleotide 3773 from adenine to an adenine/guanine mix resulting in an amino acid change at position 1226 from lysine to a lysine/asparagine mix, and/or
- z) a silent mutation in the NS3 gene at nucleotide 5391 from cytosine to thymidine, and/or
- aa) a mutation in the NS4A gene at nucleotide 6437 from cytosine to thymidine resulting in an amino acid change at position 2114 from alanine to valine, and/or
- bb) a silent mutation in the NS4B gene at nucleotide 7026 from thymidine to a thymidine/cytosine mix, and/or
- cc) a silent mutation in the NS5 gene at nucleotide 9750 from adenine to cytosine.

In another embodiments, TDV-4 comprises in addition to the three attenuating mutations the mutation s), u) and v), preferably the mutations s), u), v), c), e), x), y) and aa), even more preferably the mutations s), u), v), c), e), x), y), aa) and w), even more preferably the mutations s), u), v), c), e), x), y), aa), w), d), z), bb) and cc), and most preferably the mutations s), u), v), c), e), x), y), aa), w), d), z), bb), cc), j), k) and t). The nucleotide positions and amino acids positions of TDV-4 refer to the nucleotide sequence as shown in SEQ ID NO. 7 and amino acid sequence as shown in SEQ ID NO. 8.

In a preferred embodiment, TDV-1 has the nucleotide sequence of SEQ ID NO. 1, TDV-2 has the nucleotide sequence of SEQ ID NO. 3, TDV-3 has the nucleotide sequence of SEQ ID NO. 5, and/or TDV-4 has the nucleotide sequence of SEQ ID NO. 7. In a further preferred embodiment, TDV-1 has the amino acid sequence of SEQ ID NO. 2, TDV-2 has the amino acid sequence of SEQ ID NO. 4, TDV-3 has the amino acid sequence of SEQ ID NO. 6, and TDV-4 has the amino acid sequence of SEQ ID NO. 8. In a further preferred embodiment, TDV-1 has a nucleotide sequence encoding the amino acid sequence of SEQ ID NO. 2, TDV-2 has a nucleotide sequence encoding the amino acid sequence of SEQ ID NO. 4, TDV-3 has a nucleotide sequence encoding the amino acid sequence of SEQ ID NO. 6, and TDV-4 has a nucleotide sequence encoding the amino acid sequence of SEQ ID NO. 8.

TABLE 4

Sequences of the TDV virus strains		
SEQ ID NO.	dengue virus strain	sequence type
SEQ ID NO. 1	TDV-1	nucleotide sequence
SEQ ID NO. 2	TDV-1	amino acid sequence
SEQ ID NO. 3	TDV-2	nucleotide sequence
SEQ ID NO. 4	TDV-2	amino acid sequence
SEQ ID NO. 5	TDV-3	nucleotide sequence
SEQ ID NO. 6	TDV-3	amino acid sequence
SEQ ID NO. 7	TDV-4	nucleotide sequence
SEQ ID NO. 8	TDV-4	amino acid sequence

Thus, in a particularly preferred embodiment, the unit dose of the invention as described herein comprises the live attenuated dengue virus strains TDV-1, TDV-2, TDV-3 and TDV-4, wherein TDV-1, TDV-3 and TDV-4 are based on TDV-2 and comprise the prM and E regions of DENV-1, -3 and -4, respectively. In another particularly preferred embodiment, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid

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sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

The E protein of DENV-3 has two fewer amino acids than the E protein of DENV-2. Therefore, the nucleotides and encoded amino acid backbone of TDV-2 starting after the E region of DENV-3 at nucleotide 2374 of SEQ ID NO. 5 and amino acid 760 of SEQ ID NO. 6 are 6 nucleotides less and 2 amino acids less than the original TDV-2 nucleotide and amino acid positions, respectively.

Dengue Vaccine Composition

The present invention is in part directed to a unit dose of a dengue vaccine composition as described. The dengue vaccine composition comprises a tetravalent dengue virus composition, also referred to as dengue virus composition, and pharmaceutically acceptable excipients.

Dengue Virus Composition, Virus Concentrations and %-Concentrations

The present invention is in part directed to a unit dose of a dengue vaccine composition, wherein the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

- (i) a dengue serotype 1 preferably in a concentration of at least 3.3 log 10 pfu/0.5 mL,
- (ii) a dengue serotype 2 preferably in a concentration of at least 2.7 log 10 pfu/0.5 mL,
- (iii) a dengue serotype 3 preferably in a concentration of at least 4.0 log 10 pfu/0.5 mL, and
- (iv) a dengue serotype 4 preferably strain in a concentration of at least 4.5 log 10 pfu/0.5 mL.

The present invention is further in part directed to a unit dose of a dengue vaccine composition, wherein the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

- (i) a chimeric dengue serotype 2/1 strain in a concentration of at least 3.3 log 10 pfu/0.5 mL,
- (ii) a dengue serotype 2 strain in a concentration of at least 2.7 log 10 pfu/0.5 mL,
- (iii) a chimeric dengue serotype 2/3 strain in a concentration of at least 4.0 log 10 pfu/0.5 mL, and
- (iv) a chimeric dengue serotype 2/4 strain in a concentration of at least 4.5 log 10 pfu/0.5 mL.

Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4.

In one embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log 10 pfu/0.5 mL to 5.3 log 10 pfu/0.5 mL,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,

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- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log 10 pfu/0.5 mL to 6.0 log 10 pfu/0.5 mL, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log 10 pfu/0.5 mL to 6.5 log 10 pfu/0.5 mL.

In one such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log 10 pfu/0.5 mL to 4.9 log 10 pfu/0.5 mL,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log 10 pfu/0.5 mL to 5.7 log 10 pfu/0.5 mL, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log 10 pfu/0.5 mL to 6.2 log 10 pfu/0.5 mL.

In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log 10 pfu/0.5 mL to 4.9 log 10 pfu/0.5 mL,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log 10 pfu/0.5 mL to 5.7 log 10 pfu/0.5 mL, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log 10 pfu/0.5 mL to 5.5 log 10 pfu/0.5 mL.

In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log 10 pfu/0.5 mL to 4.1 log 10 pfu/0.5 mL,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log 10 pfu/0.5 mL to 3.6 log 10 pfu/0.5 mL,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log 10 pfu/0.5 mL to 4.7 log 10 pfu/0.5 mL, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log 10 pfu/0.5 mL to 5.3 log 10 pfu/0.5 mL.

In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log 10 pfu/0.5 mL to 3.6 log 10 pfu/0.5 mL,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log 10 pfu/0.5 mL to 4.0 log 10 pfu/0.5 mL,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log 10 pfu/0.5 mL to 4.6 log 10 pfu/0.5 mL, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log 10 pfu/0.5 mL to 5.1 log 10 pfu/0.5 mL.

In another embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $4.3 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $4.4 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $3.7 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $3.8 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.0 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $5.5 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.6 \log 10 \text{ pfu}/0.5 \text{ mL}$.

In a particularly preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $4.4 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $3.8 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $5.6 \log 10 \text{ pfu}/0.5 \text{ mL}$.

In another particularly preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $3.6 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.6 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $5.1 \log 10 \text{ pfu}/0.5 \text{ mL}$.

In another preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein the arithmetic sum of all four serotypes is less than $6.7 \log 10 \text{ pfu}/0.5 \text{ mL}$, preferably less than $5.5 \log 10 \text{ pfu}/0.5 \text{ mL}$. In certain such embodiments, the arithmetic sum of all four serotypes is at least $4.6 \log 10 \text{ pfu}/0.5 \text{ mL}$. In a preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein the arithmetic sum of all four serotypes is in the range of $4.6 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $6.7 \log 10 \text{ pfu}/0.5 \text{ mL}$, preferably in the range of $4.6 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.5 \log 10 \text{ pfu}/0.5 \text{ mL}$.

Preferably, in said embodiments the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the

nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

The present invention is in part directed to a unit dose of a dengue vaccine composition, wherein the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of at least $3.3 \log 10 \text{ pfu}/\text{dose}$,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of at least $2.7 \log 10 \text{ pfu}/\text{dose}$,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of at least $4.0 \log 10 \text{ pfu}/\text{dose}$, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of at least $4.5 \log 10 \text{ pfu}/\text{dose}$.

In one embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $3.3 \log 10 \text{ pfu}/\text{dose}$ to $5.3 \log 10 \text{ pfu}/\text{dose}$,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $2.7 \log 10 \text{ pfu}/\text{dose}$ to $5.0 \log 10 \text{ pfu}/\text{dose}$,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.0 \log 10 \text{ pfu}/\text{dose}$ to $6.0 \log 10 \text{ pfu}/\text{dose}$, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $4.5 \log 10 \text{ pfu}/\text{dose}$ to $6.5 \log 10 \text{ pfu}/\text{dose}$.

In one such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $3.3 \log 10 \text{ pfu}/\text{dose}$ to $5.0 \log 10 \text{ pfu}/\text{dose}$,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $2.7 \log 10 \text{ pfu}/\text{dose}$ to $4.9 \log 10 \text{ pfu}/\text{dose}$,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.0 \log 10 \text{ pfu}/\text{dose}$ to $5.7 \log 10 \text{ pfu}/\text{dose}$, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $4.5 \log 10 \text{ pfu}/\text{dose}$ to $6.2 \log 10 \text{ pfu}/\text{dose}$.

In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $3.3 \log 10 \text{ pfu}/\text{dose}$ to $5.0 \log 10 \text{ pfu}/\text{dose}$,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $2.7 \log 10 \text{ pfu}/\text{dose}$ to $4.9 \log 10 \text{ pfu}/\text{dose}$,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.0 \log 10 \text{ pfu}/\text{dose}$ to $5.7 \log 10 \text{ pfu}/\text{dose}$, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $4.5 \log 10 \text{ pfu}/\text{dose}$ to $5.5 \log 10 \text{ pfu}/\text{dose}$.

In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $3.3 \log 10 \text{ pfu}/\text{dose}$ to $4.1 \log 10 \text{ pfu}/\text{dose}$,

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- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $2.7 \log 10 \text{ pfu/dose}$ to $3.6 \log 10 \text{ pfu/dose}$,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.0 \log 10 \text{ pfu/dose}$ to $4.7 \log 10 \text{ pfu/dose}$, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $4.5 \log 10 \text{ pfu/dose}$ to $5.3 \log 10 \text{ pfu/dose}$.

In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $3.3 \log 10 \text{ pfu/dose}$ to $3.6 \log 10 \text{ pfu/dose}$,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $2.7 \log 10 \text{ pfu/dose}$ to $4.0 \log 10 \text{ pfu/dose}$,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.0 \log 10 \text{ pfu/dose}$ to $4.6 \log 10 \text{ pfu/dose}$, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $4.5 \log 10 \text{ pfu/dose}$ to $5.1 \log 10 \text{ pfu/dose}$.

In another embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $4.3 \log 10 \text{ pfu/dose}$ to $4.4 \log 10 \text{ pfu/dose}$,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $3.7 \log 10 \text{ pfu/dose}$ to $3.8 \log 10 \text{ pfu/dose}$,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.5 \log 10 \text{ pfu/dose}$ to $5.0 \log 10 \text{ pfu/dose}$, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $5.5 \log 10 \text{ pfu/dose}$ to $5.6 \log 10 \text{ pfu/dose}$.

In a particularly preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $4.4 \log 10 \text{ pfu/dose}$,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $3.8 \log 10 \text{ pfu/dose}$,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.5 \log 10 \text{ pfu/dose}$, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $5.6 \log 10 \text{ pfu/dose}$.

In another particularly preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

- (i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of $3.6 \log 10 \text{ pfu/dose}$,
- (ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of $4.0 \log 10 \text{ pfu/dose}$,
- (iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of $4.6 \log 10 \text{ pfu/dose}$, and
- (iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of $5.1 \log 10 \text{ pfu/dose}$.

In another preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composi-

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tion including four live attenuated dengue virus strains wherein the arithmetic sum of all four serotypes is less than $6.7 \log 10 \text{ pfu/dose}$, preferably less than $5.5 \log 10 \text{ pfu/dose}$. In certain such embodiments, the arithmetic sum of all four serotypes is at least $4.6 \log 10 \text{ pfu/dose}$. In a preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein the arithmetic sum of all four serotypes is in the range of $4.6 \log 10 \text{ pfu/dose}$ to $6.7 \log 10 \text{ pfu/dose}$, preferably in the range of $4.6 \log 10 \text{ pfu/dose}$ to $5.5 \log 10 \text{ pfu/dose}$.

Preferably, in said embodiments the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

The concentration of the different dengue viruses is preferably determined by an immuno-focus assay known in the art. For example, the concentration may be determined by an immuno-focus assay wherein serial dilutions of dengue virus are applied to monolayers of adherent cells, such as Vero cells. After a period of time which allows infectious viruses to bind to the cells and to be taken up by the cells, an overlay containing thickening agents, such as agarose or carboxymethylcellulose, is added to prevent diffusion of viruses so that progeny viruses can only infect cells adjacent to the original infected cells. After a period of incubation to allow viral replication, cells are fixed and stained using serotype-specific anti-dengue monoclonal antibodies and a secondary antibody such as an antibody labeled with alkaline phosphatase. The foci are stained by adding a suitable substrate for the enzyme attached to the secondary antibody, such as 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium phosphatase substrate. The number of plaques on the plate corresponds to the plaque forming units of the virus in the solutions applied to the cells. For example, a concentration of $1.000 \text{ pfu}/\mu\text{l}$ indicates that $1 \mu\text{l}$ of the solution applied to the cells contains enough viruses to produce 1,000 plaques in a cell monolayer.

The dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein a chimeric dengue serotype 2/1 strain, a dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain, and a chimeric dengue serotype 2/4 strain provide a total concentration in $\text{pfu}/0.5 \text{ mL}$. The term "total concentration in $\text{pfu}/0.5 \text{ mL}$ " or "total concentration in pfu/dose " is the sum of the concentrations of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), dengue serotype 2 (e.g. the dengue serotype 2 strain), the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) and the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain), preferably the sum of the concentrations of TDV-1, TDV-2, TDV-3 and TDV-4, and is defined as 100% of the dengue virus concentration as determined by pfu (plaque forming units) in 0.5 mL or in a dose.

In one embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein a dengue

serotype 1 (e.g. chimeric dengue serotype 2/1 strain), a dengue serotype 2 (e.g. dengue serotype 2 strain), a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain), and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) provide a total concentration in pfu/0.5 mL, wherein based on said total concentration the concentration of a dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is less than 10% of the total concentration, or less than 8%, or less than 6% of the total concentration, and wherein the concentration of a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) measured in pfu/0.5 mL is at least 50% or at least 60% or at least 65% of the total concentration. In one embodiment, based on said total concentration the concentration of a dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is 0.3 to 10% or 0.5 to 8% of the total concentration and the concentration of a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) measured in pfu/0.5 mL is 50% to 90% or 60% to 88% of the total concentration. This means that the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) is lower than the concentration of the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain).

In one such embodiment, the concentration of a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is at least 1% of the total concentration, and/or the concentration of a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least 6% of the total concentration, or at least 7% or 8%, 10%, 12%, 14%, 16% or 18% of the total concentration. In one such embodiment, the concentration of a dengue serotype 2 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is 1% to 7% or 2% to 6% or 2.0% to 5.0% of the total concentration, and/or the concentration of a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is 6% to 25% or 7% to 25% or 10% to 25% or 18% to 25% of the total concentration. This means that the concentration of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) is lower than the concentration of the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain).

In a preferred embodiment, the concentration of a dengue serotype 2 strain, such as TDV-2, measured in pfu/0.5 mL is less than 10% of the total concentration, preferably less than 6% or less than 2%, the concentration of a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain), such as TDV-4, measured in pfu/0.5 mL is at least 50% of the total concentration, preferably at least 65%, the concentration of a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), such as TDV-1, measured in pfu/0.5 mL is at least 1% of the total concentration, preferably between 1% and 7% or 2.0% to 5.0%, and the concentration of a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain), such as TDV-3, measured in pfu/0.5 mL is at least 6% of the total concentration, preferably between 6% and 25% or 10% to 25% or 18% to 25%.

In a further preferred embodiment, a dengue virus composition comprising a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), a dengue serotype 2 (e.g. dengue serotype 2 strain), a dengue serotype 1 (e.g. chimeric dengue serotype 2/3 strain), and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain), such as TDV-1, TDV-2, TDV-3 and TDV-4, is provided, wherein the concentration of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is at least 1% of the total concentration, preferably between 1% and 7% or 2.0% and 5.0%, the concentration of the dengue serotype 2 (e.g.

dengue serotype 2 strain) measured in pfu/0.5 mL is less than 10% of the total concentration, preferably less than 6% or less than 2% and the concentration of the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least 6% of the total concentration, preferably between 6% and 25% or 10% to 25% or 18% to 25%. It is particularly preferred that the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has the highest concentration of all four dengue serotypes.

10 In a further preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein the concentration of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is 1% to 7% of the total concentration, the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is less than 8% of the total concentration, such as in the range of 1% to 8% of the total concentration, the concentration of the dengue serotype 3 15 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least 10% of the total concentration, and the concentration of the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) measured in pfu/0.5 mL is at least 65% of the total concentration, such as in the range of 65% to 80%. In certain such embodiments, the arithmetic sum of all four serotypes is in the range of 4.6 log 10 pfu/0.5 mL to 6.7 log 10 pfu/0.5 mL, preferably in the range of 4.6 log 10 pfu/0.5 mL to 5.5 log 10 pfu/0.5 mL.

In a further preferred embodiment the dengue serotype 1 20 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and the dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 are present each in a concentration based on the total concentration in pfu/0.5 mL which is within 5%-points of each other and/or are together less than about 10% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 is preferably at least about 10% of the total concentration in pfu/0.5 mL and more preferably the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 is at least about 70% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 represents the highest concentration in the composition of all four serotypes, 25 preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 each represent lower concentrations than the concentration of serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.

30 Preferably, in said embodiments the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according

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to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

According to a further embodiment, the chimeric dengue serotype 2/4 strain, preferably TDV-4, has the highest concentration in the dengue vaccine composition, followed by the chimeric dengue serotype 2/3 strain, preferably TDV-3, followed by the chimeric dengue serotype 2/1 strain, preferably TDV-1, followed by the dengue serotype 2 strain, preferably TDV-2. It is particularly preferred that the dengue serotype 2 strain has the lowest concentration of the four strains present in the dengue vaccine composition.

Pharmaceutically Acceptable Excipients

The present invention is in part directed to a unit dose of a dengue vaccine composition, wherein the dengue vaccine composition comprises one or more pharmaceutically acceptable excipients. In one embodiment, the dengue vaccine composition comprises a non-reducing sugar, a surfactant, a protein and an inorganic salt. Preferably, the non-reducing sugar is trehalose, the surfactant is poloxamer 407, the protein is human serum albumin and the inorganic salt is sodium chloride.

In one embodiment, the unit dose of a dengue vaccine composition comprises the following pharmaceutically acceptable excipients:

from about 10% w/v to about 20% w/v α,α -trehalose dihydrate or an equimolar amount of other forms of α,α -trehalose,
from about 0.5% w/v to about 1.5% w/v poloxamer 407,
from about 0.05% w/v to about 2% w/v human serum albumin, and
from about 70 mM to 140 mM sodium chloride.

In a preferred embodiment, the lyophilized unit dose of the invention as described herein comprises the following pharmaceutically acceptable excipients:

about 15% w/v α,α -trehalose dihydrate,
about 1% w/v poloxamer 407,
about 0.1% w/v human serum albumin, and
about 100 mM sodium chloride.

In a preferred embodiment, the reconstituted unit dose of the invention as described herein comprises the following pharmaceutically acceptable excipients:

about 15% w/v α,α -trehalose dihydrate,
about 1% w/v poloxamer 407,
about 0.1% w/v human serum albumin, and
about 137 mM sodium chloride.

The human serum albumin may be a native or recombinant human serum albumin (rHSA). The poloxamer 407 may be e.g. Pluronic F127.

In one embodiment, the unit dose further comprises a buffer. The buffer may be phosphate buffered saline (PBS). The buffer may include at least one of sodium chloride (NaCl), monosodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), and potassium dihydrogen phosphate (KH_2PO_4). In a preferred embodiment, the buffer may include disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), and potassium dihydrogen phosphate (KH_2PO_4). The buffer may have a pH in the range of 7.0 to 8.5 at 25° C.

Unit Dose

The present invention is directed in part to a unit dose of a dengue vaccine composition comprising a tetravalent

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dengue virus composition as described herein and pharmaceutically acceptable excipients as described herein.

The present invention is directed in part to a unit dose of a dengue vaccine composition as described above e.g. of

- 5 (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) with a concentration of at least $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- 10 (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) with a concentration of at least $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) with a concentration of at least $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) with a concentration of at least $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$.

Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3, and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

In one embodiment, the unit dose is lyophilized. In one such embodiment, the lyophilized unit dose is obtained by subjecting a volume of 0.5 mL of the aqueous dengue vaccine composition produced by combining pharmaceutically acceptable excipients as described herein and the dengue vaccine composition as described herein comprising the four dengue virus strains, in particular TDV-1 to TDV-4, to lyophilization. In a preferred embodiment the residual moisture content as determined by Karl Fischer Determination is equal to or less than 5.0%, preferably equal to or less than 3%.

In another embodiment, the unit dose is reconstituted. The reconstituted unit dose is obtained by subjecting the lyophilized unit dose to reconstitution with a pharmaceutically acceptable diluent, preferably before administration of the dengue vaccine. In one such embodiment, reconstitution will be accomplished by adding a pharmaceutically acceptable diluent, such as water for injection, phosphate buffered saline or an aqueous sodium chloride solution, to the lyophilized unit dose. In one embodiment, an aqueous sodium chloride solution, such as a 37 mM aqueous sodium chloride solution, is added to the lyophilized unit dose for reconstitution. In one such embodiment, the lyophilized unit dose will be reconstituted with 0.3 to 0.8 mL, or 0.4 to 0.7 mL, or 0.5 mL of diluent. In a preferred embodiment, the lyophilized unit dose is reconstituted with 0.3 to 0.8 mL, 0.4 to 0.7 mL or 0.5 mL of 37 mM aqueous sodium chloride solution. In a more preferred embodiment, the lyophilized unit dose is reconstituted with 0.5 mL of 37 mM aqueous sodium chloride solution. The reconstituted unit dose can subsequently be administered subcutaneously.

It is preferred that the unit dose in lyophilized form is the final product after manufacture of the unit dose and the storage form of the unit dose, wherein the unit dose in reconstituted form is prepared before administration of the unit dose to a subject.

In one embodiment, the present invention is directed to a lyophilized unit dose of a dengue vaccine composition

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comprising upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) with a concentration of at least 3.3 log 10 pfu/0.5 mL, a dengue serotype 2 (e.g. dengue serotype 2 strain) with a concentration of at least 2.7 log 10 pfu/0.5 mL, a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) with a concentration of at least 4.0 log 10 pfu/0.5 mL, and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) with a concentration of at least 4.5 log 10 pfu/0.5 mL and pharmaceutically acceptable excipients as described herein, wherein the unit dose is preferably formulated in 0.5 mL before lyophilization. Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

In one such embodiment, the lyophilized unit dose is obtained by lyophilizing 0.5 mL of a dengue vaccine composition comprising a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of 3.3 log 10 pfu/dose to 5.0 log 10 pfu/0.5 mL, a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of 2.7 log 10 pfu/dose to 4.9 log 10 pfu/0.5 mL, a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of 4.0 log 10 pfu/dose to 5.7 log 10 pfu/0.5 mL, and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of 4.5 log 10 pfu/dose to 5.5 log 10 pfu/0.5 mL and pharmaceutically acceptable excipients as described herein. Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4.

In one such embodiment, the lyophilized unit dose is obtained by lyophilizing 0.5 mL of a dengue vaccine composition comprising a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of 3.3 log 10 pfu/0.5 mL to 3.6 log 10 pfu/0.5 mL, a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of 2.7 log 10 pfu/0.5 mL to 4.0 log 10 pfu/0.5 mL, a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of 4.0 log 10 pfu/0.5 mL to 4.6 log 10 pfu/0.5 mL, and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of 4.5 log 10 pfu/0.5 mL to 5.1 log 10 pfu/0.5 mL and pharmaceutically acceptable excipients as described herein. Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4.

In certain embodiments, the lyophilized unit dose refers to 0.5 mL before lyophilization, wherein TDV-2 and TDV-4 are present in certain relative amounts, based on the total concentration of TDV-1, TDV-2, TDV-3 and TDV-4 in pfu/0.5 mL, and the concentration of TDV-2 measured in pfu/0.5 mL is less than 10% or less than 8% or less than 6%, and the concentration of TDV-4 measured in pfu/0.5 mL is at least 50% or at least 65%. In some of these embodiments, the concentration of TDV-1 measured in pfu/0.5 mL is at

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least 1% and/or the concentration of TDV-3 measured in pfu/0.5 mL is at least 6%, 7%, 8%, 10%, 12%, 14%, 16% or at least 18%.

In certain embodiments, the reconstituted unit dose has a volume of 0.5 mL and TDV-2 and TDV-4 are present in certain relative amounts, based on the total concentration of TDV-1, TDV-2, TDV-3 and TDV-4 in pfu/0.5 mL, and the concentration of TDV-2 measured in pfu/0.5 mL is less than 10% or less than 8% or less than 6%, and the concentration of TDV-4 measured in pfu/0.5 mL is at least 50% or at least 65%. In some of these embodiments, the concentration of TDV-1 measured in pfu/0.5 mL is at least 1% and/or the concentration of TDV-3 measured in pfu/0.5 mL is at least 6%, 7%, 8%, 10%, 12%, 14%, 16% or at least 18%.

In a further preferred embodiment, the reconstituted unit dose has a volume of 0.5 mL and comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein the concentration of the dengue serotype 1 (e.g. dengue serotype 2/1 strain) measured in pfu/0.5 mL is 1% to 7% of the total concentration, the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is less than 8% of the total concentration, such as in the range of 1% to 8% of the total concentration, the concentration of the dengue serotype 3 (e.g. dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least 10% of the total concentration, and the concentration of the dengue serotype 4 (e.g. dengue serotype 2/4 strain) measured in pfu/0.5 mL is at least 65% of the total concentration, such as in the range of 65% to 80%. In certain such embodiments, the arithmetic sum of all four serotypes is in the range of 4.6 log 10 pfu/0.5 mL to 6.7 log 10 pfu/0.5 mL, preferably in the range of 4.6 log 10 pfu/0.5 mL to 5.5 log 10 pfu/0.5 mL.

In a further preferred embodiment, the reconstituted unit dose has a volume of 0.5 mL and comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and the dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 are present each in a concentration based on the total concentration in pfu/0.5 mL which is within 5%-points of each other and/or are together less than about 10% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 is preferably at least about 10% of the total concentration in pfu/0.5 mL and more preferably the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 is at least about 70% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 each represent lower concentrations than the concentration of serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.

The lyophilized unit dose reconstituted in 0.5 mL will provide the above concentrations for the four dengue serotypes. While the unit dose of a dengue vaccine composition

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as described herein refers to the concentrations of the dengue serotypes in 0.5 mL, the lyophilized unit dose can be reconstituted with other volumes of a pharmaceutically acceptable diluent, such as an aqueous sodium chloride solution, without changing the absolute virus amount administered or the ratios of the viruses to one another.

In certain embodiments, the lyophilized unit dose of the invention is prepared from a solution comprising a non-reducing sugar, a surfactant, a protein and an inorganic salt.

In certain embodiments, the lyophilized unit dose of the invention is prepared from a solution comprising trehalose, poloxamer 407, human serum albumin and sodium chloride.

In certain embodiments, the lyophilized unit dose of the invention is prepared from a solution comprising about 10% w/v to about 20% w/v α,α -trehalose dihydrate or an equimolar amount of other forms of α,α -trehalose, from about 0.5% w/v to about 1.5% w/v poloxamer 407, from about 0.05% w/v to about 2% w/v human serum albumin, and about 70 mM to about 120 mM sodium chloride.

In preferred embodiments, the lyophilized unit dose of the invention as described herein is prepared from a solution comprising about 15% w/v α,α -trehalose dihydrate, about 1% w/v poloxamer 407, about 0.1% w/v human serum albumin and about 100 mM sodium chloride.

In one embodiment, the solution from which the lyophilized unit dose is prepared further comprises a buffer. The buffer may be phosphate buffered saline (PBS). The buffer may include at least one of sodium chloride (NaCl), monosodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), and potassium dihydrogen phosphate (KH_2PO_4). In a preferred embodiment, the buffer may include disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), and potassium dihydrogen phosphate (KH_2PO_4). The buffer may have a pH in the range of about 7.0 to about 8.5 at 25° C. or a pH of about 6.8 to about 7.6 at 25° C., preferably a pH of about 7.2 at 25° C.

In preferred embodiments, the reconstituted unit dose of the invention as described herein comprising about 15% w/v α,α -trehalose dihydrate, about 1% w/v poloxamer 407, about 0.1% w/v human serum albumin and about 137 mM sodium chloride. The reconstituted unit dose may have a pH of about 7.0 to about 8.5 at 25° C., preferably a pH of about 7.2 at 25° C.

The unit dose of the invention as described herein activates multiple arms of the immune system—neutralizing antibodies, cellular immunity and anti-NS1 antibodies—in both seronegative and seropositive subject populations or in both seronegative and seropositive subjects. Thus, the unit dose of the invention as described herein protects both dengue seronegative and dengue seropositive subject populations or subjects against dengue disease.

In one embodiment, one unit dose is present in a container, preferably a vial, and said unit dose is administered to a subject after reconstitution. In one embodiment, more than one unit dose of the dengue vaccine composition may be present in a container, preferably a vial, so that with the content of one container, preferably a vial, more than one subject can be vaccinated. In one embodiment, the container comprising more than one unit doses of the invention as described herein is used for providing the reconstituted unit dose to be used in the methods of the invention as described herein.

The certain embodiments, the container comprising the unit dose of the invention is part of a kit. Thus, the invention is directed in part to a kit for preparing a reconstituted unit dose comprising a lyophilized unit dose of the present

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invention as described herein, and a pharmaceutically acceptable diluent for reconstitution.

In certain embodiments, the diluent for reconstitution provided in a container, preferably a vial, or a pre-filled syringe. In some embodiments, the diluent for reconstitution is selected from water for injection, phosphate buffered saline or an aqueous sodium chloride solution. In a preferred embodiment, the diluent for reconstitution is 30 to 40 mM sodium chloride, such as 37 mM sodium chloride.

Method of Preventing and Uses

Method of Preventing

15 The present invention is directed in part to a method of preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject. Thus, in certain embodiments the invention is directed to a method of preventing dengue disease in a subject, comprising administering to the subject a unit dose, in particular a reconstituted unit dose of the invention as described herein.

20 The present invention is directed in part to a method of preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject population. Thus, in certain embodiments the invention is directed to a method of preventing dengue disease in a subject population, comprising administering to the subject population a unit dose, in particular a reconstituted unit dose of the invention as described herein.

25 The present invention is in part directed to said method for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject population comprising administering to the subject population at least a first reconstituted unit dose of the invention as described herein, wherein certain ratios of geometric mean neutralizing antibody titers (GMTs) at day 180 or 365 after administration of said first unit dose to the subject population are achieved.

30 According to some embodiments, the geometric mean neutralizing antibody titer for dengue serotype 2 (GMT DENV-2) and the geometric mean neutralizing antibody titer for dengue serotype 4 (GMT DENV-4) when tested in at least 40, or at least 50, or at least 60 subjects at day 180 or day 365 after at least a first administration of said reconstituted unit dose of the invention as described herein, and optionally a second administration of a reconstituted unit dose of the invention as described herein 90 days after said first administration, provide a ratio of GMT DENV-2:GMT DENV-4 of not more than 50, or not more than 40, or not more than 30, or not more than 20. In some of these embodiments, the ratio of GMT DENV-2:GMT DENV-1 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose, and/or the ratio of GMT DENV-2:GMT DENV-3 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose.

35 The present invention is in part directed to said method for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject comprising administering to the subject at least a first reconstituted unit dose of the invention as described herein, wherein certain ratios of neutralizing antibody titers at day 180 or 365 after administration of said first unit dose to the subject are achieved. According to some embodiments, the neutralizing antibody titer for dengue serotype 2 and the neutralizing antibody titer for dengue serotype 4 at day 180 or day 365 after at least a first administration of the reconstituted unit dose of the invention as described herein, and optionally a second

administration of a reconstituted unit dose of the invention as described herein 90 days after said first administration, provide a ratio of neutralizing antibody titer for DENV-2: neutralizing antibody titer for GMT DENV-4 of not more than 50, or not more than 40, or not more than 30, or not more than 20. In some of these embodiments, the ratio of the neutralizing antibody titers of DENV-2:DENV-1 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose, and/or the ratio of the neutralizing antibody titers of DENV-2:DENV-3 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose.

The geometric mean neutralizing antibody titers (GMTs) of a subject population or the neutralizing antibody titers of a subject are determined in accordance with the microneutralization test disclosed herein, for example according to the method described in Example 2. Without wishing to be bound to any theory, it is presently understood that a method inducing a more balanced immune response due to the administration of the reconstituted unit dose of the invention as described herein, in terms of less differences between the geometric mean neutralizing antibody titers (GMTs) against the four dengue serotypes or the neutralizing antibody titers against the four dengue serotypes, is beneficial to the subject or subject population to be vaccinated. In particular, it is understood that a much greater response to any one of the four serotypes, such as to DENV-2 in comparison to the other serotypes, is less beneficial.

The present invention is in part directed to said method for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject or subject population wherein the method provides a seropositivity rate in a subject population of at least 50 subjects including the administration of two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population are seronegative to all dengue serotypes at baseline. In certain such embodiments, at least 80% of the subject population are seropositive for all four dengue serotypes at least one month after administration of the first unit dose, such as at day 30, and/or at least 80% of the subject population are seropositive for all four dengue serotypes before or at the time of the administration of the second unit dose, such as at day 90, and/or at least 80%, or at least 85%, or at least 90%, or at least 95% of the subject population are seropositive for all four dengue serotypes after the administration of the second unit dose, such as at day 120, and/or at least 80%, or at least 85%, or at least 90% of the subject population are seropositive for all four dengue serotypes after the administration of the second unit dose, such as at day 270.

The present invention is in part directed to said method for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject or subject population wherein the method provides a seropositivity rate in a subject population of at least 100 subjects including administration of two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population comprises from 20% to 40% subjects who are seronegative to all dengue serotypes and from 60% to 80% subjects who are seropositive to at least one dengue serotype at base line, wherein at day 120 and/or day 270 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 10%-points and/or wherein at day 120 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population

and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 5%-points.

The present invention is in part directed to a method of preventing virologically confirmable dengue disease in a subject or subject population comprising administering to the subject or subject population a reconstituted unit dose of a tetravalent dengue virus composition including four live, attenuated dengue serotypes, in particular the virus strains as described herein.

The present invention is in part directed to a method of preventing virologically confirmable dengue disease with hospitalization in a subject or subject population comprising administering to the subject or subject population a reconstituted unit dose of a tetravalent dengue virus composition including four live, attenuated dengue serotypes, in particular the virus strains as described herein.

In certain embodiments, the invention is directed to said methods, wherein said dose unit comprises a tetravalent dengue virus composition including four live attenuated dengue serotypes, in particular the virus strains described herein wherein the serotypes have certain concentrations as described herein with respect to the virus composition and unit dose such as:

- 25 (i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,
- (ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log 10 pfu/0.5 mL to 4.9 log 10 pfu/0.5 mL,
- (iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log 10 pfu/0.5 mL to 5.7 log 10 pfu/0.5 mL, and
- (iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log 10 pfu/0.5 mL to 5.5 log 10 pfu/0.5 mL.

In preferred such embodiments, the subject or subject population is of 2 to 17 years of age, such as 4 to 16 years of age, and preferably less than 9 years of age. In other preferred embodiments, the subject or subject population is 4-5 years of age, 6-11 years of age or 12-16 years of age.

In certain embodiments, the invention is directed to said methods, wherein said unit dose upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent has a concentration of 3.3 log 10 pfu/0.5 mL to 3.6 log 10 pfu/0.5 mL for dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), has a concentration of 2.7 log 10 pfu/0.5 mL to 4.0 log 10 pfu/0.5 mL for dengue serotype 2 (e.g. dengue serotype 2 strain), has a concentration of 4.0 log 10 pfu/0.5 mL to 4.6 log 10 pfu/0.5 mL for dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) and has a concentration of 4.5 log 10 pfu/0.5 mL to 5.1 log 10 pfu/0.5 mL for dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain). In preferred such embodiments, the subject or subject population is of 2 to 17 years of age, such as 4 to 16 years of age, and preferably less than 9 years of age. In other preferred embodiments, the subject or subject population is 4-5 years of age, 6-11 years of age or 12-16 years of age.

In certain embodiments, the invention is directed to said methods, wherein the concentration of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is 1% to 7% of the total concentration, the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is less than 8% of the total concentration, such as in the range of 1% to 8% of the total concentration, the concentration of the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in

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pfu/0.5 mL is at least 10% of the total concentration, and the concentration of the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) measured in pfu/0.5 mL is at least 65% of the total concentration, such as in the range of 65% to 80%. In certain such embodiments, the arithmetic sum of all four serotypes is in the range of 4.6 log 10 pfu/0.5 mL to 6.7 log 10 pfu/0.5 mL, preferably in the range of 4.6 log 10 pfu/0.5 mL to 5.5 log 10 pfu/0.5 mL. Preferably, in said embodiments the subject or subject population is of 2 to 17 years of age, such as 4 to 16 years of age, and even more preferably less than 9 years of age. In other preferred embodiments, the subject or subject population is 4-5 years of age, 6-11 years of age or 12-16 years of age.

In a further preferred embodiment, the invention is directed to said methods, wherein the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and the dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 are present each in a concentration based on the total concentration in pfu/0.5 mL which is within 5%-points of each other and/or are together less than about 10% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 is preferably at least about 10% of the total concentration in pfu/0.5 mL and more preferably the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 is at least about 70% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 each represent lower concentrations than the concentration of serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.

Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered by subcutaneous injection. According to some of these embodiments, the subcutaneous injection is administered to the arm, preferably to the deltoid region of the arm.

In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose is administered to a subject of unknown serostatus and/or wherein no test has been carried out to determine whether the subject is

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seropositive or seronegative before the unit dose as described herein is administered.

In certain embodiments, the invention is directed to said methods, wherein the subject or subject population is seronegative to all dengue serotypes.

In certain embodiments, the invention is directed to said methods, wherein two unit doses of the invention as described herein are administered. In some embodiments the two unit doses are administered within 12 months or more, 10 or within six months, or within three months, and optionally at least 4 weeks apart such as at day 0 and day 90 or at day 1 and day 90. According to some of these embodiments, a further third unit dose of the invention as described herein is administered after the second administration. Such a third 15 administration may act as a booster and may be administered between 6 to 12 months after the first administration, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months after the second administration.

In certain embodiments, the method of the invention comprises or consists of a single unit dose of the invention being administered.

In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject or subject population that is seronegative with respect to all dengue serotypes. In other embodiments, the subject or subject population is seropositive with respect to at least one dengue serotype.

In certain embodiments, the invention is directed to said methods, wherein the unit dose of the invention as described herein is administered to a subject or subject population from a dengue endemic region. In some of these embodiments, the subject or subject population is from Singapore, 25 Dominican Republic, Panama, Philippines, Colombia, Puerto Rico or Thailand, in particular from Singapore, Dominican Republic, Panama, or Philippines. In a preferred embodiment, the subject or subject population is from Asia Pacific or from Latin America. In some other of these embodiments, the subject or subject population is from Thailand, Sri Lanka, Philippines, Panama, Nicaragua, Dominican Republic, Colombia or Brazil. In other embodiments, the subject or subject population is from a dengue non-endemic region. Such a subject population or such a 30 subject may be vaccinated according to the present invention in the context of traveling to a dengue endemic region. In certain embodiments, the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject or subject population that is from a dengue endemic region or a dengue non-endemic region.

In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject or subject population of 2 to 60 years of age. In some 35 embodiments, the subjects or subject population are adults of 18 to 60 years.

In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to children and adolescents of 2 to 17 years of age. In some 40 embodiments, the subjects or subject population are less than 9 years of age, or less than 4 years of age. In some embodiments, the subjects or subject population are from 2 to 9 years of age, or from 2 to 5 years of age, or from 4 to 45 9 years of age or from 6 to 9 years of age. In other embodiment, the subject or subject population is 4 to 16 years of age. In some such embodiments, the subject or

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subject population is 4-5 years of age, 6-11 years of age or 12-16 years of age. Optionally, the subject or subject population is seronegative with respect to all dengue serotypes.

In certain embodiments, the invention is directed to said methods, wherein the unit dose of the invention as described herein is administered to a pediatric subject or pediatric subject population of less than 2 years of age, preferably of 2 months to 2 years or 2 months to 1.5 years or 2 months to 1 year. According to some of these embodiments, the pediatric subject or pediatric subject population is seronegative and from a dengue endemic region.

In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered to a pediatric subject or pediatric subject population of less than 2 years of age, preferably of 2 months to 2 years or 2 months to 1.5 years or 2 months to 1 year, preferably by subcutaneous injection. According to some of these embodiments, the pediatric subject or pediatric subject population is seronegative and from a dengue endemic region.

In a certain embodiments, the invention is directed to said methods, wherein the subject or subject population is 4-5 years of age and from Asia Pacific, 6-11 years of age and from Asia Pacific, or 12-16 years of age and from Asia Pacific. In other embodiments, the subject or subject population is 4-5 years of age and from Latin America, 6-11 years of age and from Latin America, or 12-16 years of age and from Latin America.

In a certain embodiments, the invention is directed to said methods, wherein the subject or subject population is 4-5 years of age and seropositive for at least 1 dengue serotype, 6-11 years of age and seropositive for at least 1 dengue serotype, or 12-16 years of age and seropositive for at least 1 dengue serotype. In other embodiments, the subject or subject population is 4-5 years of age and seronegative for all dengue serotypes, 6-11 years of age and seronegative for all dengue serotypes, or 12-16 years of age and seronegative for all dengue serotypes.

In a certain embodiments, the invention is directed to said methods, wherein the subject or subject population is from Asia Pacific or Latin America and seropositive for at least one dengue serotype at baseline. In other embodiments, the subject or subject population is from Asia Pacific or Latin America and seronegative for at all dengue serotype at baseline.

In certain embodiments, the invention is directed to said methods, wherein the subject or subject population is from Asia Pacific, seropositive for at least one dengue serotype at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In other embodiments, the subject or subject population is from Asia Pacific, seronegative for all dengue serotypes at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In yet other embodiments, the subject or subject population is from Latin America, seropositive for at least one dengue serotype at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In other embodiments, the subject or subject population is from America, seronegative for all dengue serotypes at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age.

In certain embodiments, the invention is directed to said methods, wherein the subject or subject population had prior vaccination against Yellow Fever. In other embodiments, the subject or subject population had prior vaccination against Japanese Encephalitis. In yet other embodiments, the subject or subject population had no prior vaccination against Yellow Fever. In other embodiments, the subject or subject population had no prior vaccination against Japanese

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Encephalitis. Prior vaccination indicates a vaccination prior to 30 days after a second administration, such as within 4 months after the first administration, with the reconstituted unit dose as described herein. For example for vaccine efficacy (VE) as determined in Example 6 from 30 days post-second vaccination, a prior vaccination of Yellow Fever is defined as a Yellow Fever vaccination occurring before 30 days post-second vaccination. In certain embodiments, the subject or subject population received Denvaxia within the 10 administration regimen as described herein or within 4.5 years after administration of the first dose.

Particularly unbalanced titers of neutralizing antibodies against the four dengue serotypes are observed in seronegative populations or subjects after administration of the 15 commercially available dengue vaccine. The present invention shows that in particular seronegative subjects show a more balanced immune response to the four dengue serotypes after administration of the reconstituted unit dose of the invention as described herein. It is therefore contemplated that the unit dose of the invention as described herein 20 and methods of the present invention as described herein may provide a more robust immune response in a subject population including both seropositive and seronegative subjects.

The present invention is directed in part to a method of preventing virologically confirmable dengue disease in a 25 subject comprising administering to the subject a tetravalent dengue virus composition including four dengue virus strains representing serotype 1, serotype 2, serotype 3 and 30 serotype 4, wherein the virus strains are optionally live, attenuated dengue virus strains.

The present invention is directed in part to a method of preventing virologically confirmable dengue disease in a 35 subject consisting of administering to the subject a tetravalent dengue virus composition including four dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4, wherein the virus strains are optionally live, attenuated dengue virus strains.

In certain embodiments, the invention is directed to said 40 methods, wherein there is no step of determining the serostatus of the subject at baseline, in other words, said methods do not comprise a determination of a previous dengue infection of the subject at baseline before the administration of the tetravalent dengue virus composition. In particular, 45 such methods are safe and effective. Thus, in certain such embodiments, the subject has not been tested for the presence a previous dengue infection.

In certain embodiments, the invention is directed to said 50 methods, wherein the vaccine administration is safe irrespective of whether there is a determination that the subject had a previous dengue infection before the administration of the tetravalent dengue virus composition. In particular, such methods are also effective.

In certain embodiments, the invention is directed to said 55 methods, wherein the method is safe and/or effective.

In certain embodiments, the invention is directed to said 60 methods, wherein the composition includes at least one chimeric dengue virus. In certain such embodiments, the invention is directed to said methods, wherein the composition includes at least one non-chimeric dengue virus and at least one chimeric dengue virus, in particular a chimeric dengue serotype 2/1 strain and a dengue serotype 2 strain and a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain. The details of the composition 65 are described above.

Therefore, in certain embodiments, the invention is directed to said methods having a vaccine efficacy, prefer-

ably a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In embodiments, the invention is directed to said methods having a vaccine efficacy, preferably a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose as described herein or placebo is administered at least once, until 15 months after the first administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, more than 40%, more than 50%, more than 55%, more than 60%, more than 65%, more than 70% or more than 72%. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

In certain embodiments, the invention is directed to said methods having a vaccine efficacy, preferably a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain embodiments, the invention is directed to said methods having a vaccine efficacy, preferably a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose as described herein or placebo is administered at least once, until 15 months after the first administration of the administration schedule. In certain such embodiments, the vaccine efficacy is more than 40%, more than 50%, more than 55%, more than 60%, more than 65%, more than 70%, more than 75%, more than 78%, more than 79% or about 80%. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

In certain embodiments, the invention is directed to said methods having a vaccine efficacy, preferably a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization with a 2-sided 95% confidence interval, wherein the lower bound is more than 0%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration

until at least 18 months after the second administration. In certain such embodiments, the lower bound is more than 10%, more than 20%, more than 30%, more than 40%, more than 50%, more than 55%, more than 60%, more than 65%, more than 70% or is more than 80%, or more than 90%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes in seronegative subjects with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, more than 40%, more than 50%, or is more than 55%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes in seronegative subjects of more than 30%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the combined vaccine efficacy against all four dengue serotypes in seronegative subjects is more than 40%, more than 50%, is more than 60%, more than 65%, or is more than 70%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 4 to 5 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, more than 40%, is more than 45%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 4 to 5 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the combined vaccine efficacy against all four dengue serotypes is more than 40%, more than 50%, more than 60%, more than 65%, or is more than 70%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 6 to 11 years of age at the time of randomization and irrespective of serostatus at baseline, wherein

said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, or is more than 70%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 6 to 11 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 75%, or is more than 80%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 12 to 16 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 68%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 12 to 16 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 75%, or is more than 80%.

In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 1 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, or is more than 50%.

In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 1 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at

least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the vaccine efficacy against dengue serotype 1 is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 70%.

10 In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 2 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at
15 least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration
20 schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50, is more than 60, is more than 70, is more than 80, or is more than 90%.

25 In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 2 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the 35 vaccine efficacy against dengue serotype 2 is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80, or is more than 90%.

In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 40 3 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said 45 unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such 50 embodiments, the lower bound is more than 30%, is more than 40%.

In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 3 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the vaccine efficacy against dengue serotype 3 is more than 40%, is more than 50%, is more than 55%, or is more than 60%.

65 In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all

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four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 70%, or is more than 75%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes of more than 30%, when measured against placebo in a subject population of at least 2,000 healthy subjects, healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 90%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seropositive at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 70%, or is more than 80%.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes of more than 30%, when measured against placebo in a subject population of at least 2,000 healthy subjects, healthy subjects being seropositive at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 90%.

In certain embodiments, the invention is directed to said methods having a relative risk, preferably a combined relative risk against all four serotypes, with a 2-sided 95% confidence interval, wherein the upper bound is less than 0.75, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the last administration of the administration schedule until at least 12 months after the last administration of the administration schedule.

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second administration until at least 12 months after the second administration. In certain such embodiments, the upper bound is less than 0.70, less than 0.65, less than 0.60, less than 0.55, less than 0.50, less than 0.45, less than 0.40, less than 0.35, less than 0.30 or less than 0.28. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

In certain embodiments, the invention is directed to said methods having a relative risk, preferably a combined relative risk against all four serotypes, of less than 0.70, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the relative risk is less than 0.65, less than 0.60, less than 0.55, less than 0.50, less than 0.45, less than 0.40, less than 0.35, less than 0.30, less than 0.25 or less than 0.23. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

In certain embodiments, the invention is directed to said methods, wherein virologically confirmable dengue disease occurs in less than 2.5% of the subjects, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or at least 18 months after the second administration. In certain such embodiments, virologically confirmable dengue disease occurs in less than 2.0% of the subjects, less than 1.5% of the subjects, less than 1.0% of the subjects, less than 0.8% of the subjects, or less than 0.6% of the subjects. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 61.0%, or more than 65.0 or more than 70.0% or more than 72.0% when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) from endemic irrespective of serostatus at baseline and being selected from the group consisting of 4 to 16 year old subjects at the time of randomization, wherein said unit dose or said placebo is administered at least twice within 6 months or less, about 30 days after the last administration of the administration schedule until at least 12 or 13 months after the last administration of the administration schedule.

In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes of more than 66%, or of more than 70%, or of more than 75%, or of more than 77%, or of more than 80.0%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) from endemic areas irrespective of serostatus at baseline and being selected from the group consisting of 4 to 16 year old subjects at the time of randomization, wherein said unit dose or said placebo is administered at least twice within 6 months or less, about 30 days after the last administration of the administration

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schedule until at least 12 months or 13 month after the last administration of the administration schedule.

In certain embodiments, the invention is directed to said methods, wherein the combined vaccine efficacy against all four serotypes is measured about 30 days after the last administration of the administration schedule until 12 or 13 months after the last administration of the administration schedule.

In certain embodiments, the invention is directed to said methods, wherein said unit dose or said placebo is administered at twice within three months, in particular at about day 1 and about day 90, and wherein the combined vaccine efficacy against all four serotypes is measured 30 days after the second administration until 12 or 13 months after the second administration of the administration schedule.

In certain embodiments, the invention is directed to said methods, wherein said methods are effective and safe. In some of these embodiments, the subject or subject population is under 9 years of age, under 4 years of age, or under 2 years of age or from 2 to 9 years of age, or from 2 to 5 years of age, or from 4 to 9 years of age or from 6 to 9 years of age. Optionally the subject is seronegative with respect to all dengue serotypes.

In certain embodiments, the invention is directed to said methods, wherein said methods having a relative risk for virologically confirmed dengue with hospitalization of 1 or less, or 0.8 or less, or 0.6 or less, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects). In some of these embodiments, the subject or subject population is under 9 years of age, under 4 years of age, or under 2 years of age or from 2 to 9 years of age, or from 2 to 5 years of age, or from 4 to 9 years of age or from 6 to 9 years of age. Optionally the subject is seronegative with respect to all dengue serotypes.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are 4 to 16 years of age. In some of such embodiments, the healthy subjects of the subject population are 4 to 5 years of age, 6 to 11 years of age, or 12 to 16 years of age.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are defined as being healthy in view of the exclusion criteria specified in Example 6.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are from Asia Pacific or Latin America.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are seropositive with respect to at least one serotype. In other embodiments, the healthy subjects of the subject population are seronegative with respect to all serotypes.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are 4-5 years of age and from Asia Pacific, 6-11 years of age and from Asia Pacific, or 12-16 years of age and from Asia Pacific. In other embodiments, the healthy subjects of the subject population are 4-5 years of age and from Latin America, 6-11 years of age and from Latin America, or 12-16 years of age and from Latin America.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are 4-5 years of age and seropositive for at least 1 dengue serotype, 6-11 years of age and seropositive for at least 1 dengue serotype, or 12-16 years of age and seropositive for at least 1 dengue serotype. In other embodiments, the healthy subjects of the subject population are 4-5 years of

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age and seronegative for all dengue serotypes, 6-11 years of age and seronegative for all dengue serotypes, or 12-16 years of age and seronegative for all dengue serotypes.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are from Asia Pacific or Latin America and seropositive for at least one dengue serotype at baseline. In other embodiments, the healthy subjects of the subject population are from Asia Pacific or Latin America and seronegative for all dengue serotype at baseline.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are from Asia Pacific, seropositive for at least one dengue serotype at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In other embodiments, the healthy subjects of the subject population are from Asia Pacific, seronegative for all dengue serotypes at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age.

In yet other embodiments, the healthy subjects of the subject population are from Latin America, seropositive for at least one dengue serotype at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In other embodiments, the healthy subjects of the subject population are from America, seronegative for all dengue serotypes at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population had prior vaccination against Yellow Fever. In other embodiments, the healthy subjects of the subject population had no prior vaccination against Yellow Fever. Prior vaccination indicates a vaccination prior to the first vaccination with the reconstituted unit dose as described herein. For example for vaccine efficacy (VE) as determined in Example 6 from 30 days post-second vaccination, a prior vaccination of Yellow Fever is defined as a Yellow Fever vaccination occurring before 30 days post-second vaccination.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population had prior vaccination against Japanese Encephalitis. In other embodiments, the healthy subjects of the subject population had no prior vaccination against Japanese Encephalitis.

In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population received Denvaxia within the administration regimen as described herein or within 4.5 years after administration of the first dose. In certain embodiments, the invention is directed to said methods, wherein the occurrence of vaccine related serious adverse events is less than 0.1%.

In certain embodiments, the invention is directed to said methods, wherein the occurrence of vaccine related unsolicited adverse events occurring within 4 weeks of administration is less than 2%.

In certain embodiments, the invention is directed to said methods, wherein the occurrence of vaccine related solicited adverse events occurring within 2 weeks of administration is less than 35%.

In certain embodiments, the invention is directed to said methods, wherein the occurrence of vaccine related solicited local reactions occurring within 1 weeks of administration is less than 40%.

In certain embodiments, the invention is directed to said methods, wherein the method does not increase the risk of

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virologically-confirmed dengue with hospitalization in the individual, such as in a seronegative individual.

Unit Dose for Use in a Method of Preventing Dengue Disease

The present invention is directed in part to the composition or unit dose of the invention as described herein for use in a method of preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject.

The present invention is directed in part to the composition or unit dose of the invention as described herein for use in a method of preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject population.

Any method described herein above under the heading "Method of preventing" is to be understood to be also disclosed as unit dose for use in such a method of preventing dengue disease in a subject or subject population irrespective of being expressly stated below.

The present invention is directed in part to a reconstituted unit dose of a dengue vaccine composition as described herein for use in a method of preventing virologically confirmable dengue disease in a subject comprising administering at least a first unit dose of the dengue vaccine composition to the subject, wherein the dengue vaccine composition is a tetravalent dengue virus composition including four dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, optionally wherein the dengue virus strains are live, attenuated dengue virus strains and/or comprise chimeric dengue viruses and/or at least one non-chimeric dengue virus, and wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent

- (i) dengue serotype 1 has a concentration of at least 3.3 log 10 pfu/0.5 mL and optionally to 5.0 log 10 pfu/0.5 mL,
- (ii) dengue serotype 2 has a concentration of at least 2.7 log 10 pfu/0.5 mL and optionally to 4.9 log 10 pfu/0.5 mL,
- (iii) dengue serotype 3 has a concentration of at least 4.0 log 10 pfu/0.5 mL and optionally to 5.7 log 10 pfu/0.5 mL, and
- (iv) dengue serotype 4 has a concentration of at least 4.5 log 10 pfu/0.5 mL and optionally to 6.2 log 10 pfu/0.5 mL.

The present invention is directed in part to a reconstituted unit dose of a dengue vaccine composition as described herein for use in a method of preventing virologically confirmable dengue disease in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, wherein the dengue vaccine composition is a tetravalent dengue virus composition including four dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, optionally wherein the dengue virus strains are live, attenuated, and wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent

- (v) dengue serotype 1 has a concentration of at least 3.3 log 10 pfu/0.5 mL and optionally to 5.0 log 10 pfu/0.5 mL,

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(vi) dengue serotype 2 has a concentration of at least 2.7 log 10 pfu/0.5 mL and optionally to 4.9 log 10 pfu/0.5 mL,

(vii) dengue serotype 3 has a concentration of at least 4.0 log 10 pfu/0.5 mL and optionally to 5.7 log 10 pfu/0.5 mL, and

(viii) dengue serotype 4 has a concentration of at least 4.5 log 10 pfu/0.5 mL and optionally to 6.2 log 10 pfu/0.5 mL.

In certain embodiments, the invention is directed to a reconstituted unit dose of a dengue vaccine composition for use in a method of preventing virologically confirmable dengue disease in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, wherein the dengue vaccine composition is a tetravalent dengue virus composition including four dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, optionally wherein the dengue virus strains are live, attenuated, wherein the subject is under 9 years of age and/or when the serostatus of the subject is unknown or seronegative and wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent

(i) dengue serotype 1 has a concentration of at least 3.3 log 10 pfu/0.5 mL and optionally to 5.0 log 10 pfu/0.5 mL,

(ii) dengue serotype 2 has a concentration of at least 2.7 log 10 pfu/0.5 mL and optionally to 4.9 log 10 pfu/0.5 mL,

(iii) dengue serotype 3 has a concentration of at least 4.0 log 10 pfu/0.5 mL and optionally to 5.7 log 10 pfu/0.5 mL, and

(iv) dengue serotype 4 has a concentration of at least 4.5 log 10 pfu/0.5 mL and optionally to 6.2 log 10 pfu/0.5 mL.

In certain embodiments, the reconstituted unit dose is administered to a subject of unknown serostatus and/or wherein no test has been carried out to determine whether the subject is seropositive or seronegative before the unit dose as described herein is administered.

In certain embodiments, the subject is under 9 years of age and/or the serostatus of the subject is unknown or seronegative. In certain such embodiments, the subject is under 9 years of age and the serostatus of the subject is unknown or seronegative, preferably seronegative.

In certain embodiments, the method is safe. In certain such embodiments, the subject is under 9 years of age or from 4 years of age and/or the serostatus of the subject is unknown or seronegative. In certain such embodiments, the subject is from 4 years of age and the serostatus of the subject is unknown or seronegative, preferably seronegative.

In certain embodiments, the method is effective. In certain such embodiments, the subject is under 9 years of age and/or the serostatus of the subject is unknown or seronegative. In certain such embodiments, the subject is under 9 years of age and the serostatus of the subject is unknown or seronegative, preferably seronegative.

In certain embodiments, the dengue serotype 1 and the dengue serotype 2 are present each in a concentration based on the total concentration in pfu/0.5 mL which is within 5%-points of each other and/or are together less than about 10% of the total concentration in pfu/0.5 mL. In certain such embodiments, the dengue serotype 3 is at least about 10% of

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the total concentration in pfu/0.5 mL and/or the dengue serotype 4 is at least about 70% of the total concentration in pfu/0.5 mL.

In certain embodiments, the dengue serotype 4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 and dengue serotype 2 each represent lower concentrations than the concentration of serotype 3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.

In certain embodiments, the composition includes at least one chimeric dengue virus. In certain such embodiments, the composition includes at least one non-chimeric dengue virus and at least one chimeric dengue virus.

In certain embodiments, the subject is seronegative to all dengue serotypes at baseline and/or is from 4 years of age, optionally to 60 years of age. In certain such embodiments, the subject is 4 to 16 years of age, under 9 years of age, from 2 years of age to under 9 years of age, from 4 years of age to under 9 years of age, 4 to 5 years of age, 6 to 11 years of age, or 12 to 16 years of age. In other embodiments, the subject is seropositive to at least one dengue serotypes at baseline and/or is from 4 years of age, optionally to 60 years of age. In certain such embodiments, the subject is 4 to 16 years of age, under 9 years of age, from 2 years of age to under 9 years of age, from 4 years of age to under 9 years of age, 4 to 5 years of age, 6 to 11 years of age, or 12 to 16 years of age.

In certain embodiments, the method does not comprise a determination of a previous dengue infection in the subject before the administration of the first unit dose of the tetravalent dengue virus composition. Thus, in certain embodiments, the subject has not been tested for the presence a previous dengue infection.

In certain embodiments, the dengue serotype 1 is a chimeric dengue serotype 2/1 strain, the dengue serotype 2 is a non-chimeric dengue serotype 2 strain, the dengue serotype 3 is a chimeric dengue serotype 2/3 strain and the dengue serotype 4 is a chimeric dengue serotype 2/4 strain and the dengue serotype 1 has the amino acid sequence of SEQ ID NO. 2, the dengue serotype 2 has the amino acid sequence of SEQ ID NO. 4, the dengue serotype 3 has the amino acid sequence of SEQ ID NO. 6, and the dengue serotype 4 has the amino acid sequence of SEQ ID NO. 8.

In certain embodiments, the unit dose further comprises from about 10% w/v to about 20% w/v trehalose dihydrate or an equimolar amount of other forms of α,α -trehalose, from about 0.5% w/v to about 1.5 w/v poloxamer 407, from about 0.05% w/v to about 2% w/v human serum albumin, and from about 70 mM to 140 mM sodium chloride when measured in 0.5 mL. In certain such embodiments, the unit dose comprises about 15 (w/v) α,α -trehalose dihydrate, about 1% (w/v) poloxamer 407, about 0.1% (w/v) human serum albumin, and about 100 mM sodium chloride when measured in 0.5 mL.

In certain embodiments, the method is for preventing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).

In certain embodiments, the subject is from a dengue endemic region. In other embodiments, the subject is from a dengue non-endemic region.

In certain embodiments, the subject is from Asia Pacific or Latin America.

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In certain embodiments, the reconstituted unit dose provides a seropositivity rate when it is administered to a subject population of at least 50 subjects in two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population are seronegative to all dengue serotypes at baseline. In certain such embodiments, at least 80% of the subject population are seropositive for all four dengue serotypes at least one month after administration of the first unit dose, such as at day 30, and/or at least 80% of the subject population are seropositive for all four dengue serotypes before or at the time of the administration of the second unit dose, such as at day 90, and/or at least 80%, or at least 85% or at least 90%, or at least 95% of the subject population are seropositive for all four dengue serotypes after the administration of the second unit dose, such as at day 120, and/or at least 80%, or at least 85%, or at least 90% of the subject population are seropositive for all four dengue serotypes after the administration of the second unit dose, such as at day 270. In certain such embodiments, at least 80% of the subject population are seropositive for all four dengue serotypes at least one month after administration of the first unit dose, such as at day 30, and before or at the time of the administration of the second unit dose, such as at day 90, and after the administration of the second unit dose, such as at day 120 and at day 270.

In certain embodiments, the reconstituted unit dose provides a seropositivity rate, when it is administered to a subject population of at least 100 subjects in two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population comprises from 20% to 40% subjects who are seronegative to all dengue serotypes and from 60% to 80% subjects who are seropositive to at least one dengue serotype at base line, wherein at day 120 and/or day 270 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 10%-points and/or wherein at day 120 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 5%-points.

In certain particular embodiments, the invention is directed to a dengue vaccine composition as described herein for use in a method of preventing virologically confirmable dengue disease in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, and wherein the dengue vaccine composition is a tetravalent dengue virus composition including four live, attenuated dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4 wherein the attenuated dengue virus strains comprise chimeric dengue viruses and at least one non-chimeric dengue virus, and wherein the dengue serotype 1 and the dengue serotype 2 are present each in a concentration based on the total concentration in pfu/0.5 mL which is within 5%-points of each other and/or are together less than about 10% of the total concentration in pfu/0.5 mL.

In certain embodiments, the method does not comprise a determination of a previous dengue infection of the subject before the administration of the first unit dose of the tetravalent dengue virus composition and wherein the method

is safe and effective. Thus, in certain embodiments, the subject has not been tested for the presence a previous dengue infection.

In certain embodiments, the dengue serotype 3 is at least about 10% of the total concentration in pfu/0.5 mL and/or the dengue serotype 4 is at least about 70% of the total concentration in pfu/0.5 mL.

In certain embodiments, the dengue serotype 4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 and dengue serotype 2 each represent lower concentrations than the concentration of serotype 3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.

In certain embodiments, the dengue serotype 1 is a chimeric dengue serotype 2/1 strain, the dengue serotype 2 is a non-chimeric dengue serotype 2 strain, the dengue serotype 3 is a chimeric dengue serotype 2/3 strain and the dengue serotype 4 is a chimeric dengue serotype 2/4 strain and the dengue serotype 1 has the amino acid sequence of SEQ ID NO. 2, the dengue serotype 2 has the amino acid sequence of SEQ ID NO. 4, the dengue serotype 3 has the amino acid sequence of SEQ ID NO. 6, and the dengue serotype 4 has the amino acid sequence of SEQ ID NO. 8.

In certain embodiments, in the unit dose upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent

- (i) dengue serotype 1 has a concentration of 3.3 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,
- (ii) dengue serotype 2 has a concentration of 2.7 log 10 pfu/0.5 mL to 4.9 log 10 pfu/0.5 mL,
- (iii) dengue serotype 3 has a concentration of 4.0 log 10 pfu/0.5 mL to 5.7 log 10 pfu/0.5 mL, and
- (iv) dengue serotype 4 has a concentration of 4.5 log 10 pfu/0.5 mL to 6.2 log 10 pfu/0.5 mL, and optionally the composition further comprises about 15% (w/v) α,α' -trehalose dihydrate, about 1% (w/v) poloxamer 407, about 0.1% (w/v) human serum albumin, and about 100 mM sodium chloride when measured in 0.5 mL.

In certain embodiments, the unit doses are administered to the deltoid region of the arm.

In certain embodiments, the composition is administered without determining the serostatus of the subject at baseline and wherein the administration is safe and effective regardless of the serostatus at base line.

In certain embodiments, the subject is seronegative to all dengue serotypes at baseline and/or is from 4 years of age, optionally to 60 years of age. In certain such embodiments, the subject is 4 to 16 years of age, under 9 years of age, from 2 years of age to under 9 years of age, from 4 years of age to under 9 years of age, 4 to 5 years of age, 6 to 11 years of age, or 12 to 16 years of age. In particular the subject may be under 9 years of age and seronegative to all four dengue serotypes at baseline. In other embodiments, the subject is seropositive to at least one dengue serotypes at baseline and/or is from 4 years of age, optionally to 60 years of age. In certain such embodiments, the subject is 4 to 16 years of age, under 9 years of age, from 2 years of age to under 9 years of age, from 4 years of age to under 9 years of age, 4 to 5 years of age, 6 to 11 years of age, or 12 to 16 years of age. In particular the subject may be under 9 years of age and seropositive to at least one dengue serotypes at baseline. In certain preferred embodiments, the subject is 4 to 5 years of age, 6 to 11 years of age or 12 to 16 years of age.

In certain embodiments, the method is for preventing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).

In certain embodiments, the subject is from a dengue endemic region or from a dengue non-endemic region.

In certain embodiments, the subject is from Asia Pacific or Latin America.

In certain embodiments, the composition provides a seropositivity rate when it is administered to a subject population of at least 50 subjects in two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population are seronegative to all dengue serotypes at baseline, in particular wherein at least one month after administration of the first unit dose, such as at day 30, at least 80% of the subject population are seropositive for all four dengue serotypes, and/or at least 80% of the subject population are seropositive for all four dengue serotypes before or at the time of the administration of the second unit dose, such as at day 90, and/or at least 80%, or at least 85% or at least 90%, or at least 95% of the subject population are seropositive for all four dengue serotypes after the administration of the second unit dose, such as at day 120, and/or at least 80%, or at least 85%, or at least 90% of the subject population are seropositive for all four dengue serotypes after the administration of the second unit dose, such as at day 270.

In certain embodiments, the composition provides a seropositivity rate, when it is administered to a subject population of at least 100 subjects in two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population comprises from 20% to 40% subjects who are seronegative to all dengue serotypes and from 60% to 80% subjects who are seropositive to at least one dengue serotype at base line, wherein at day 120 and/or day 270 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 10%-points and/or wherein at day 120 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 5%-points.

The present invention is in part directed to the unit dose of the invention as described herein for use in a method of preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject population comprising administering to the subject population at least a first reconstituted unit dose of the invention as described herein, wherein certain ratios of geometric mean neutralizing antibody titers (GMTs) at day 180 or 365 after administration of said first unit dose to the subject population are achieved. According to some embodiments, the geometric mean neutralizing antibody titer for dengue serotype 2 (GMT DENV-2) and the geometric mean neutralizing antibody titer for dengue serotype 4 (GMT DENV-4) when tested in at least 40, or at least 50, or at least 60 subjects at day 180 or day 365 after at least a first administration of said reconstituted unit dose of the invention as described herein, and optionally a second administration of a reconstituted unit dose of the invention as described herein 90 days after said first administration, provide a ratio of GMT DENV-2:GMT DENV-4 of not more than 50, or not more than 40, or not more than 30, or not more than 20. In some of these embodiments, the ratio of GMT DENV-2:GMT DENV-1 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose, and/or the ratio of GMT DENV-2:GMT DENV-3 is not more than 20,

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or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose.

The present invention is in part directed to the unit dose of the invention as described herein for use in a method of preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject comprising administering to the subject at least a first reconstituted unit dose of the invention as described herein, wherein certain ratios of neutralizing antibody titers at day 180 or 365 after administration of said first unit dose to the subject are achieved. According to some embodiments, the neutralizing antibody titer for dengue serotype 2 and the neutralizing antibody titer for dengue serotype 4 at day 180 or day 365 after at least a first administration of the reconstituted unit dose of the invention as described herein, and optionally a second administration of a reconstituted unit dose of the invention as described herein 90 days after said first administration, provide a ratio of neutralizing antibody titer for DENV-2: neutralizing antibody titer for GMT DENV-4 of not more than 50, or not more than 40, or not more than 30, or not more than 20. In some of these embodiments, the ratio of the neutralizing antibody titers of DENV-2:DENV-1 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose, and/or the ratio of the neutralizing antibody titers of DENV-2:DENV-3 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose.

The geometric mean neutralizing antibody titers (GMTs) of a subject population or the neutralizing antibody titers of a subject are determined in accordance with the microneutralization test disclosed herein, for example according to the method described in Example 2.

In certain embodiments the invention is directed to the reconstituted unit dose of the invention as described herein for said uses, wherein said unit dose is administered by subcutaneous injection. According to some of these embodiments the subcutaneous injection is administered to the arm, preferably to the deltoid region of the arm.

In certain embodiments the invention is directed to a reconstituted unit dose of the invention as described herein for said uses, wherein the subject or subject population is seronegative to all dengue serotypes.

In certain embodiments the invention is directed to a reconstituted unit dose of the invention as described herein for said uses, wherein a single unit dose of the invention as described herein is administered.

In certain embodiments the invention is directed to a reconstituted unit dose of the invention as described herein for said uses, wherein two reconstituted unit doses of the invention as described herein are administered. In some embodiments, the two reconstituted unit doses are administered within 12 months or more, or within six months, or within three months, such as at day 0 and day 90 or at day 1 and day 90. According to some of these embodiments, a third reconstituted unit dose of the invention as described herein may be administered after the second administration. Such a third administration may act as a booster and may be administered between 6 to 12 months after the first administration, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months after the second administration.

In certain embodiments the invention is directed to a reconstituted unit dose of the invention as described herein for said uses, wherein the reconstituted unit dose of the invention as described herein is administered at most in two doses or in one dose.

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In certain embodiments of the invention the subject is seronegative with respect to all dengue serotypes. In certain embodiments of the invention the subject is seronegative with respect to all dengue serotypes and the reconstituted unit dose is administered to the seronegative subject by subcutaneous injection.

In certain other embodiments of the invention the subject is seropositive with respect to at least one dengue serotype.

In certain embodiments the invention is directed to the reconstituted unit dose of the invention as described herein for said uses, wherein the reconstituted unit dose of the invention as described herein is administered to a subject or subject population from a dengue endemic region. In some of these embodiments, the subject or subject population is from Singapore, Dominican Republic, Panama, Philippines, Colombia, Puerto Rico or Thailand, in particular from Singapore, Dominican Republic, Panama, or Philippines. In other embodiments, the subject or subject population is from a dengue non-endemic region. Such a subject population or such a subject may be vaccinated according to the invention in the context of traveling to a dengue-endemic region. In certain embodiments, the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject or subject population from a dengue endemic region or from a dengue non-endemic region.

In some embodiments the invention is directed to the unit dose of the invention as described herein for said uses, wherein the subject or subject population is of 2 to 60 years of age or 18 to 60 years of age. In certain embodiments, the subject or subject population is of 1 to 17 years of age, or less than 9 years of age, or less than 4 years of age or less than 2 years of age. According to some of these embodiments the subject or subject population is seronegative and from a dengue-endemic region.

In certain embodiments, the invention is directed to the reconstituted unit dose of the invention as described herein for said uses, wherein the unit dose of the invention as described herein is administered to a pediatric subject or pediatric subject population of less than 2 years of age, preferably of 2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject or pediatric subject population is seronegative and from a dengue endemic region.

In certain embodiments, the invention is directed to the reconstituted unit dose of the invention as described herein for said uses, wherein the reconstituted unit dose is administered subcutaneously to a pediatric subject or pediatric subject population of less than 2 years of age, preferably of 2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject or pediatric subject population is seronegative and from a dengue endemic region.

The unit dose for use in the methods described above may be any unit dose of a dengue vaccine composition as described above under the headings "Unit dose" or "Dengue vaccine composition" and comprise any dengue virus strain as described above under the heading "Dengue virus strain".

Use for the Manufacture of a Medicament for Preventing Dengue Disease

The present invention is directed in part to the use of a unit dose of the invention as described herein for the manufac-

ture of a medicament for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject.

The present invention is directed in part to the use of a unit dose of the invention as described herein for the manufacture of a medicament for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject population.

Any method described herein above under the heading "Method of preventing" is to be understood to be also disclosed as the use of a unit dose for the manufacture of a medicament for preventing dengue disease in a subject or subject population with such a method irrespective of being expressly stated below.

The present invention is in part directed to the use of a unit dose of the invention as described herein for the manufacture of a medicament for preventing dengue disease in a subject population, comprising administering to the subject population at least a first reconstituted unit dose of the invention as described herein, wherein certain ratios of geometric mean neutralizing antibody titers (GMTs) at day 180 or 365 after administration of said first unit dose to the subject population are achieved. According to some embodiments, the geometric mean neutralizing antibody titer for dengue serotype 2 (GMT DENV-2) and the geometric mean neutralizing antibody titer for dengue serotype 4 (GMT DENV-4) when tested in at least 40, or at least 50, or at least 60 subjects at day 180 or day 365 after at least a first administration of said reconstituted unit dose of the invention as described herein, and optionally a second administration of a reconstituted unit dose of the invention as described herein 90 days after said first administration, provide a ratio of GMT DENV-2:GMT DENV-4 of not more than 50, or not more than 40, or not more than 30, or not more than 20. In some of these embodiments, the ratio of GMT DENV-2:GMT DENV-1 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose, and/or the ratio of GMT DENV-2:GMT DENV-3 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose.

The present invention is in part directed to the use of a unit dose of the invention as described herein for the manufacture of a medicament for preventing dengue disease in a subject, comprising administering to the subject at least a first reconstituted unit dose of the invention as described herein, wherein certain ratios of neutralizing antibody titers at day 180 or 365 after administration of said first unit dose to the subject are achieved. According to some embodiments, the neutralizing antibody titer for dengue serotype 2 and the neutralizing antibody titer for dengue serotype 4 at day 180 or day 365 after at least a first administration of the reconstituted unit dose of the invention as described herein, and optionally a second administration of a reconstituted unit dose of the invention as described herein 90 days after said first administration, provide a ratio of neutralizing antibody titer for DENV-2: neutralizing antibody titer for GMT DENV-4 of not more than 50, or not more than 40, or not more than 30, or not more than 20. In some of these embodiments, the ratio of the neutralizing antibody titers of DENV-2:DENV-1 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose, and/or the ratio of the neutralizing antibody titers of DENV-2:DENV-3 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose.

In some embodiments, the geometric mean neutralizing antibody titers (GMTs) of a subject population or the neutralizing antibody titers of a subject are determined in accordance with the microneutralization test disclosed herein, for example according to the method described in Example 2.

In certain embodiments the invention is directed to said uses, wherein the reconstituted unit dose of the invention as described herein is administered by subcutaneous injection. 10 According to some of these embodiments the subcutaneous injection is administered to the arm, preferably to the deltoid region of the arm.

In certain embodiments the invention is directed to said uses, wherein one reconstituted unit dose of the invention as 15 described is administered,

In certain embodiments the invention is directed to said uses, wherein two reconstituted unit doses of the invention as described herein are administered. In one embodiment, the two unit doses are administered within 12 months or 20 more, or within six months, or within three months, such as at day 0 and day 90 or at day 1 and day 90. According to some of these embodiments a third unit dose of the invention as described herein may be administered after the second administration. Such a third administration may act as a booster and may be administered between 6 to 12 months after the first administration, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months after the second administration.

In certain embodiments of the invention the subject is 30 seronegative with respect to all dengue serotypes.

In certain other embodiments of the invention the subject is seropositive with respect to at least one dengue serotype.

In certain embodiments the invention is directed to said uses, wherein the reconstituted unit dose is administered to 35 the seronegative subject by subcutaneous injection.

In certain embodiments the invention is directed to said uses, wherein the reconstituted unit dose is administered to a subject of unknown serostatus and/or wherein no test has been carried out to determine whether the subject is sero- 40 positive or seronegative before the unit dose is administered.

In certain embodiments the invention is directed to said uses, wherein the reconstituted unit dose of the invention as described herein is administered to a subject or subject population from a dengue endemic region. In some of these 45 embodiments, the subject or subject population is from Singapore, Dominican Republic, Panama, Philippines, Colombia, Puerto Rico or Thailand, in particular from Singapore, Dominican Republic, Panama, or Philippines. In other embodiments, the subject or subject population is from a dengue non-endemic region. Such a subject population or 50 subject may be vaccinated according to the invention in the context of traveling to a dengue endemic region. In certain embodiments, the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject or subject population from a dengue endemic region or from a dengue non-endemic region.

In certain embodiments the invention is directed to said uses, wherein the subject is of 2 to 60 years of age or 18 to 60 years of age. In certain embodiments the subject is 1 to 17 years of age, or less than 9 years of age, or less than 4 years of age or less than 2 years of age. According to some of these embodiments the subject is seronegative and from a dengue-endemic region.

In certain embodiments, the invention is directed to said 65 uses, wherein the unit dose of the invention as described herein is administered to a pediatric subject or pediatric subject population of less than 2 years of age, preferably of

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2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject or pediatric subject population is seronegative and from a dengue endemic region.

In certain embodiments, the invention is directed to said uses, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a pediatric subject or pediatric subject population of less than 2 years of age, preferably of 2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject or pediatric subject population is seronegative and from a dengue endemic region.

Method of Stimulating an Immune Response and Uses

Method of Stimulating an Immune Response

In certain embodiments the invention is directed to a method for stimulating an immune response, preferably a balanced immune response, to all four dengue serotypes in a subject, comprising administering to the subject a reconstituted unit dose of the invention as described herein.

The present invention is in part directed to a method for stimulating an immune response to all four serotypes of dengue virus in a subject, comprising administering to the subject a reconstituted unit dose of the invention as described herein by subcutaneous injection.

In certain embodiments, the invention is directed to said method, wherein the immune response to all four serotypes of dengue virus is balanced.

In certain embodiments, the invention is directed to said method, wherein the reconstituted unit dose is administered by subcutaneous injection to the arm, preferably to the deltoid region of the arm.

In certain embodiments, the invention is directed to said method, wherein the subject is seronegative to all dengue serotypes.

In certain embodiments, the invention is directed to said method, wherein two reconstituted unit doses of the invention as described herein are administered. In some embodiments, the two reconstituted doses are administered within 12 months or more, or within six months, or within three months, such as at day 0 and day 90 or at day 1 and day 90. According to some of these embodiments, a third unit dose of the invention as described herein is administered between 6 and 12 months after the administration of said first unit dose, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months after the second administration.

In certain embodiments, the invention is directed to said method, wherein the unit dose of the invention as described herein is administered to a subject from a dengue endemic region. In some of these embodiments, the subject is from Singapore, Dominican Republic, Panama, Philippines, Colombia, Puerto Rico or Thailand, in particular from Singapore, Dominican Republic, Panama, or Philippines. In other embodiments, the subject is from a dengue non-endemic region. Such a subject may be subject to a vaccination according to the invention in the context of traveling to a dengue endemic region. In certain embodiments, the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject that is from a dengue endemic region or a dengue non-endemic region.

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In certain embodiments, the invention is directed to said method, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject that is seronegative with respect to all dengue serotypes. In other embodiments, the subject is seropositive with respect to at least one dengue serotype.

In certain embodiments, the invention is directed to said method, wherein the neutralizing antibody titers of the subject when tested at day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, provide a ratio of not more than 50, or not more than 40, or nor more than 30, or not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4. In certain embodiments, said neutralizing antibody titers of the subject further provide a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 1, and/or a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 3.

In certain embodiments, the invention is directed to said method, wherein the unit dose of the invention as described herein is administered to a subject of 2 to 60 years of age or 18 to 60 years of age. In certain embodiments the subject is 1 to 17 years of age, or less than 9 years of age, or less than 4 years of age or less than 2 years of age. According to some of these embodiments the subject is seronegative and from a dengue-endemic region.

In certain embodiments, the invention is directed to said method, wherein the unit dose of the invention as described herein is administered to a pediatric subject of less than 2 years of age, preferably of 2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject is seronegative and from a dengue endemic region.

In certain embodiments, the invention is directed to said method, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a pediatric subject of less than 2 years of age, preferably of 2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject is seronegative and from a dengue endemic region.

Unit Dose for Use in a Method of Stimulating an Immune Response

50 The present invention is in part directed to the reconstituted unit dose of the invention as described herein for use in a method for stimulating an immune response to all four serotypes of dengue virus in a subject.

The present invention is in part directed to the reconstituted unit dose of the invention as described herein for use in a method for stimulating an immune response to all four serotypes of dengue virus in a subject, wherein a reconstituted unit dose of the invention as described herein is administered to the subject, preferably by subcutaneous injection.

In certain embodiments, the invention is directed to the reconstituted unit dose of the invention as described herein for said use, wherein the immune response to all four serotypes of dengue virus is balanced.

65 In certain embodiments, the invention is directed to the reconstituted unit dose of the invention as described herein for said use, wherein the reconstituted unit dose is admin-

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istered by subcutaneous injection to the arm, preferably to the deltoid region of the arm.

In certain embodiments, the invention is directed the reconstituted unit dose of the invention as described herein for said use, wherein the subject is seronegative to all dengue serotypes.

In certain embodiments, the invention is directed to the unit dose of the invention as described herein for said use, wherein two reconstituted unit doses of the invention as described herein are administered. In some embodiments, the two reconstituted unit doses are administered within 12 months or more, or within six months, or within three months, such as at day 0 and day 90 or at day 1 and day 90. According to some of these embodiments, a third reconstituted unit dose is administered 6 to 12 months after the administration of the first reconstituted unit dose, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months after the second administration.

In certain embodiments, the invention is directed to the unit dose of the invention as described herein for said use, wherein the subject is from a dengue endemic region. In other embodiments, the subject is from a dengue non-endemic region. Such a subject may be subject to a vaccination according to the invention in the context of traveling to a dengue endemic region. In certain embodiments, the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject that is from a dengue endemic region or a dengue non-endemic region.

In certain embodiments, the invention is directed to the unit dose of the invention as described herein for said use, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject that is seronegative with respect to all dengue serotypes. In other embodiments, the subject is seropositive with respect to at least one dengue serotype.

In certain embodiments, the invention is directed to the unit dose of the invention as described herein for said use, wherein the neutralizing antibody titers of the subject when tested at day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, provide a ratio of not more than 50, or not more than 40, or nor more than 30, or not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4. In certain embodiments, said neutralizing antibody titers of the subject further provide a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 1, and/or a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 3.

In certain embodiments, the invention is directed to the reconstituted unit dose of the invention as described herein for said use, wherein the unit dose of the invention as described herein is administered to a subject of 2 to 60 years of age or 18 to 60 years of age. In certain embodiments the subject is 1 to 17 years of age, or less than 9 years of age, or less than 4 years of age or less than 2 years of age. According to some of these embodiments the subject is seronegative and from a dengue-endemic region.

In certain embodiments, the invention is directed to the reconstituted unit dose of the invention as described herein for said use, wherein the unit dose of the invention as described herein is administered to a pediatric subject of less than 2 years of age, preferably of 2 months to 2 years of age

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or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject is seronegative and from a dengue endemic region.

In certain embodiments, the invention is directed to the reconstituted unit dose of the invention as described herein for said use, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a pediatric subject of less than 2 years of age, preferably of 2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject is seronegative and from a dengue endemic region.

Use for the Manufacture of a Medicament for Stimulating an Immune Response

The present invention is in part directed to the use of the reconstituted unit dose of the invention as described herein for the manufacture of a medicament for stimulating an immune response to all four serotypes of dengue virus in a subject. In one embodiment a reconstituted unit dose of the invention as described herein is administered by subcutaneous injection.

In certain embodiments, the invention is directed to said use, wherein the immune response to all four serotypes of dengue virus is balanced.

In certain embodiments, the invention is directed to said use, wherein the reconstituted unit dose is administered by subcutaneous injection to the arm, preferably to the deltoid region of the arm.

In certain embodiments, the invention is directed the reconstituted unit dose of the invention as described herein for said use, wherein the subject is seronegative to all dengue serotypes.

In certain embodiments, the invention is directed to said use, wherein two reconstituted unit doses of the invention as described herein are administered. In some embodiments, the two reconstituted unit doses are administered within 12 months or more, or within six months, or within three months, such as at day 0 and day 90 or at day 1 and day 90. According to some of these embodiments, a third reconstituted unit dose is administered 6 to 12 months after the administration of the first reconstituted unit dose, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months after the second administration.

In certain embodiments, the invention is directed to said use, wherein the subject is from a dengue endemic region. In other embodiments, the subject is from a dengue non-endemic region. Such a subject may be subject to a vaccination according to the invention in the context of traveling to a dengue endemic region. In certain embodiments, the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject that is from a dengue endemic region or a dengue non-endemic region.

In certain embodiments, the invention is directed to said use, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject that is seronegative with respect to all dengue serotypes. In other embodiments, the subject is seropositive with respect to at least one dengue serotype.

In certain embodiments, the invention is directed to said use, wherein the neutralizing antibody titers of the subject when tested at day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, provide a ratio of not more than 50, or not

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more than 40, or nor more than 30, or not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4. In certain embodiments, said neutralizing antibody titers of the subject further provide a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 1, and/or a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 3.

In certain embodiments, the invention is directed to said use, wherein the reconstituted unit dose of the invention as described herein is administered to a subject of 2 to 60 years of age or 18 to 60 years of age. In certain embodiments the subject is 1 to 17 years of age, or less than 9 years of age, or less than 4 years of age or less than 2 years of age. According to some of these embodiments the subject is seronegative and from a dengue-endemic region.

In certain embodiments, the invention is directed to said use, wherein the unit dose of the invention as described herein is administered to a pediatric subject of less than 2 years of age, preferably of 2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject is seronegative and from a dengue endemic region.

In certain embodiments, the invention is directed to said use, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a pediatric subject of less than 2 years of age, preferably of 2 months to 2 years of age or 2 months to 1.5 years of age or 2 months to 1 year of age. According to some of these embodiments, the pediatric subject is seronegative and from a dengue endemic region.

Method for Determining the Titer of Neutralizing Antibodies

The present invention is directed in part to a method for determining the titer of neutralizing antibodies against each of dengue serotypes 1, 2, 3 and 4 in a blood serum sample, the method comprising the steps of:

- (a) seeding cells from a dengue-susceptible cell line on 96-well assay plates and culturing the cells for a culture period;
- (b) preparing serial dilutions of the blood serum sample;
- (c) separately mixing the serially diluted blood serum samples prepared in step (b) with dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4 to obtain separate mixtures for each dengue serotype and incubating the separate mixtures;
- (d) adding the separate mixtures prepared in (c) to the cells seeded and cultured in step (a) and incubating the cells with the separate mixtures;
- (e) providing an overlay for the inoculated cells and incubating the cells for an incubation period of 40 to 75 hours;
- (f) determining the number of plaques in each well and comparing the number of plaques in each well to a control to determine the level of neutralizing antibodies against each of dengue serotypes 1, 2, 3 and 4.

In one embodiment, different incubation periods are used in step (e) for the mixtures of different dengue serotypes. In some embodiments, the incubation period for mixtures of dengue serotype 4 is shorter than the incubation period for mixtures of dengue serotypes 1, 2 and 3, for example the incubation period for mixtures of dengue serotype 4 is less than 50 hours, preferably 46 ± 2 hours. In some embodiments,

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the incubation period for mixtures of dengue serotype 2 is longer than the incubation period for mixtures of dengue serotypes 1, 3 and 4, for example the incubation period for mixtures of dengue serotype 2 is between 60 and 70 hours, preferably 70 ± 2 hours.

In one embodiment, the dengue-susceptible cell line used in step (a) is selected from Vero cells, LLC-MK2 cells and BHK-21 cells. In some embodiments, the culture period of the cells is 12 to 36 hours.

In one embodiment, in step (c) the dengue serotype 1 is DENV-1 strain 16007, dengue serotype 2 is DENV-2 strain 16681, dengue serotype 3 is DENV-3 strain 16562 and dengue serotype 4 is DENV-4 strain 1036.

In one embodiment, the separate mixtures in step (c) are incubated overnight at a temperature of 2 to $8^\circ C$.

In one embodiment, the overlay in step (e) is selected from the group consisting of methylcellulose, carboxymethylcellulose and agarose. In some embodiments, the cells with the overlay are incubated at a temperature of $33^\circ C$. to $35^\circ C$.

In one embodiment, the number of plaques in each well is determined using serotype-specific anti-dengue monoclonal antibodies.

In one embodiment, the invention is directed to a method for determining the titer of neutralizing antibodies against each of dengue serotypes 1, 2, 3 and 4 in a blood serum sample, the method comprising the steps of:

- (a) seeding Vero cells on 96-well assay plates and culturing the Vero cells for a period of 20 to 30 hours;
- (b) preparing serial dilutions of the serum sample;
- (c) separately mixing the serially diluted serum samples with dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4 to prepare separate mixtures and incubating the separate mixtures overnight at a temperature of 2 to $8^\circ C$;
- (d) incubating the cells seeded and cultured in step (a) with the separate mixtures prepared in step (c) in separate wells for 90 to 120 minutes;
- (e) providing a methylcellulose overlay for the inoculated cells and incubating the cells for an incubation period of 40 to 75 hours at $34^\circ C$;
- (f) determining the number of plaques in each well using serotype-specific anti-dengue monoclonal antibodies and comparing the number of plaques in each well to a control to determine the level of neutralizing antibodies against each of dengue serotypes 1, 2, 3 and 4.

In one embodiment, the invention is directed to the use of said method for determining the dengue serostatus of a subject before vaccination with a dengue virus vaccine or for analyzing a subjects immune response after vaccination with a dengue virus vaccine.

EXAMPLES

The following Examples are included to demonstrate certain aspects and embodiments of the invention as described in the claims. It should be appreciated by those of skill in the art, however, that the following description is illustrative only and should not be taken in any way as a restriction of the invention.

Example 1

Preparation of the Dengue Virus Strains

The methods used to generate the chimeric dengue strains TDV-1, -3 and -4 were standard molecular cloning and DNA

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engineering methods and are described in Huang et al. (2003) J. Virology 77(21): 11436-11447. The following well-known methods were used to construct and introduce the prM-E genes of dengue serotypes 1, 3 and 4 into the TDV-2 backbone: Reverse-transcriptase PCR (RT-PCR), PCR, restriction enzyme digestion, DNA fragment ligation, bacterial transformations by electroporation, plasmid DNA preparations, in vitro transcription by T7 RNA polymerase, and transfection of Vero cells by electroporation.

After growing and purifying the different dengue serotypes separately as described in Huang et al. (2013) PLOS Neglected Dis. 7(5):e2243, they are mixed in the concentrations provided in Table 5. The mixture of dengue serotypes is present in a dengue vaccine composition and combined with a composition of pharmaceutically acceptable excipients resulting in a dengue vaccine composition comprising 15% w/v α,α trehalose dihydrate, 1% w/v poloxamer 407, 0.1% w/v human serum albumin and 100 mM sodium chloride. The dengue vaccine composition is lyophilized and represents a lyophilized unit dose of TDV. The lyophilized unit dose is reconstituted with 37 mM aqueous sodium chloride solution and the reconstituted unit dose comprises 15% w/v α,α trehalose dihydrate, 1% w/v poloxamer 407, 0.1% w/v human serum albumin and 137 mM sodium chloride.

Example 2

Microneutralization Test

Immunogenicity was measured by a microneutralization assay to each one of the four dengue serotypes with titers defined as the dilution resulting in a 50% reduction in plaque values (MNT50). Briefly, on day 1 Vero cells were seeded on 96-well assay plates in DMEM and 10% FBS at a density of 2.5×10^5 cells/ml and incubated at 37° C. for 24 hours. On day 2 serial dilutions of the heat-inactivated antibody-containing test and control sera samples (dilutions range 1:10 to 1:20480) were prepared and mixed with a constant concentration of dengue viruses, in particular DENV-1 strain 16007, DENV-2 strain 16681, DENV-3 strain 16562 and DENV-4 strain 1036, (target 60-80 pfu/well) in a 96 well microtiter plate and incubated overnight at 2-8° C. to enable the neutralization of the virus by the antibodies present in the sera. After the incubation the mixture of virus and antibodies was transferred onto the 96 well plates with Vero cells and the plates were incubated at 37° C. for 90-120 minutes to infect the Vero cells. A 1% methylcellulose overlay in DMEM was applied to the plate to restrict spread of progeny virus and the plate was incubated for 46-70 hours at 34° C. depending on the Dengue serotype:

DENV1—66±2 hours

DENV2—70±2 hours

DENV3—66±2 hours

DENV4—46±2 hours

After the incubation the cells were washed twice with PBS and fixed by adding cold methanol and incubating for 60 minutes at a temperature of ≤-20° C. After fixing the plates were dried and washed three times with washing buffer (1× PBS, pH 7.4 with 0.5% Tween), before 50 µl of serotype-specific anti-dengue monoclonal antibodies in blocking solution (2.5% nonfat dry milk in PBST) per well were added and incubated with the cells for 18±4 hours at 2-8° C.

The monoclonal antibodies were made as described in Gentry et al. (1982) Am. J. Trop. Med. Hyg. 31, 548-555; Henchal et al. (1985) Am. J. Trop. Med. Hyg. 34, 162-169;

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and Henchal et al. (1982) Am. J. Trop. Med. Hyg. 31(4): 830-6). Briefly, the anti-DENV-1 HBD was made against dengue 1 strain Hawaii, Envelope, the anti-DENV-2 was made against dengue 2 strain New Guinea C, Envelope, isotype 1, the anti-DENV-3 HBD was made against dengue 3 strain H87, Envelope, isotype 2A, and the anti-DENV-4 HBD was made against dengue 4 strain H241, Envelope, isotype 2A.

After incubation, the plates were washed three times with washing buffer and 50 µl of a secondary peroxidase labelled goat anti-mouse IgG (H+L) (KPL Cat #074-1806) in blocking solution was added and incubated for 90 to 120 minutes at 37° C. Then the plates were washed three times with washing buffer and 50 µl of precipitant substrate (2-amino-9-ethyl carbazole (AEC) tablet in 2.5 ml DMSO, 47.5 ml 50 mM acetate buffer and 250 µl hydrogen peroxide) were added and the mixture was incubated for 20 minutes at room temperature. Finally, the substrate was removed, the plates were rinsed with dH₂O and dried.

Sample titers are calculated using the linear regression method and reported as MNT50 titers for each sample. Clinical data are reported as a geometric mean titer for all the individual MNT50 titers in each treatment group. Briefly, the number of infectious foci in each well was counted and the titer of neutralizing antibodies was determined by comparing the percent reduction of infectious foci centers in wells containing antibody (test samples) in comparison to wells containing virus alone. The MNT50 was calculated using the following linear regression equation:

$$\text{MNT50} = 10^{[(50-c)/m]} \text{ where } c = \text{y intercept of regression line and } m = \text{slope of regression line}$$

Each test sample was tested in triplicates and the titer was calculated from the average of the triplicates. A schematic drawing of the steps performed in this test is provided in FIG. 2.

Example 3

Clinical Trial Comparing Two Different Unit Doses

A descriptive Phase II, double-blind, randomized, and controlled trial in 351 subjects aged 21 to 45 years living in Singapore was performed comparing two different formulations of a tetravalent dengue vaccine. Subjects were randomized (1:1), stratified by baseline dengue serostatus, into two treatment groups: group 1 received one subcutaneous (SC) dose of HD-TDV and group 2 received one subcutaneous dose of TDV. The dengue vaccine formulations were prepared as described in Example 1. The concentration of the four dengue serotypes in the HD-TDV vaccine (high dose tetravalent dengue vaccine) and in the TDV vaccine (corresponding to the unit dose of the invention as described herein) is outlined in Table 5.

TABLE 5

Unit doses used in the trial.	
Comparative unit dose (HD-TDV)	0.5 mL comprising TDV-1, TDV-2, TDV-3 and TDV-4 containing 3.2×10^4 PFU, 1.3×10^5 PFU, 2.5×10^5 PFU, and 4×10^5 PFU, respectively. Administered by subcutaneous injection on day 1.
Example 1 unit dose (TDV)	0.5 mL comprising TDV-1, TDV-2, TDV-3 and TDV-4 containing 2.5×10^4 PFU, 6.3×10^3 PFU, 3.2×10^4 PFU, and 4×10^5 PFU, respectively. Administered by subcutaneous injection on day 1.

Immunogenicity was evaluated at Days 15, 30, 90, 180, and 365 post-vaccination as geometric mean titers (GMTs) and seropositivity rates. Immunogenicity of the vaccines against each of the four dengue serotypes was assessed using a microneutralization assay, with titers corresponding to the dilution resulting in a 50% reduction in plaque reduction (MNT50) as described in Example 2. Primary immunogenicity endpoints were reported in terms of geometric mean titers (GMTs) of neutralizing antibodies, and seropositivity rates (which were defined as percentages of subjects with reciprocal neutralizing titers ≥ 10 for each of the DENV serotypes) in the overall trial population. As a secondary endpoint, GMTs and seropositivity rates were analyzed by dengue baseline seropositivity status. Seropositive at baseline was defined as being seropositive for at least one DENV serotype, whereas seronegative at baseline was defined as being seronegative for all four DENV serotypes.

Solicited and unsolicited adverse events (AEs) were assessed by severity and causality.

a) Seropositivity

Dengue seropositivity is based on the result of the microneutralization test (MNT) described in Example 2 and is defined as a reciprocal neutralizing antibody titer for one or more dengue serotype at baseline. The baseline seropositivity rate for each dengue serotype is defined as the percentage of seropositive subjects for the given dengue serotype and was derived from the neutralizing antibodies titers of the dengue serotypes as measured in the subjects before administration of the first unit dose. The seropositivity rate at day 180 or day 365 is defined as the percentage of seropositive subjects and was derived from the neutralizing antibodies titers of the dengue serotypes as measured in the subjects 180 and 365 days after administration of the first unit dose, respectively.

In total, 187 subjects (53.6%) were seropositive, based on MNT50, for at least one dengue serotype at baseline: 48.7% were seropositive for DENV-1, 49.0% for DENV-2, 45.2% for DENV-3, and 41.2% for DENV-4. The seropositive status and rate at baseline of the two different vaccination groups is shown in Table 6.

TABLE 6

Serostatus and seropositivity rate at baseline		
	Comparative unit dose	Example 1 unit dose
Baseline seropositivity status (count of participants)		
Seropositive for at least one dengue serotype	92	95
Seronegative for all dengue serotypes	83	80
Baseline seropositivity rate for each serotype (percentage of participants)		
TDV-1	48.6	48.6
TDV-2	47.4	50.3
TDV-3	44.0	46.3
TDV-4	41.7	40.6

Seropositivity rates increased to Day 30 after administration of the unit doses, and remained high through to Day 365 for each of the four serotypes (FIG. 3). For the overall trial population, the percentages of subjects who were seropositive for DENV-1 and DENV-3 were similar in the HD-TDV and TDV groups, whereas higher post-baseline seropositivity rates were seen for the HD-TDV group against DENV-2,

and for the TDV group against DENV-4 (FIG. 3B). In general, higher seropositivity rates were seen in subjects already seropositive at baseline than in seronegative subjects. Seropositivity rates rose to nearly 100% against all four dengue serotypes in the seropositive vaccine groups by Day 30, and remained at this level through to Day 365; no difference was seen between HD-TDV and TDV (FIG. 3A). In the seronegative group, the seropositivity rates increased more gradually to a peak at Day 30, with limited decline until Day 365. The rates were consistently higher for HD-TDV than TDV against DENV-2, but were higher for TDV than HD-TDV against DENV-4, through to Day 365 (FIG. 3A).

TABLE 7

Seropositivity rate at day 180		
	Comparative unit dose	Example 1 unit dose
Overall number of participants analyzed	166	163
Seropositivity rate at day 180 (95% Confidence Interval)		
Day 180, TDV-1	93.4 (88.5 to 96.6)	89.6 (83.8 to 93.8)
Day 180, TDV-2	98.2 (94.8 to 99.6)	92.6 (87.5 to 96.1)
Day 180, TDV-3	88.0 (82.0 to 92.5)	82.2 (75.5 to 87.7)
Day 180, TDV-4	78.9 (71.9 to 84.9)	84.0 (77.5 to 89.3)

TABLE 8

Seropositivity rate at day 365		
	Comparative unit dose	Example 1 unit dose
Overall number of participants analyzed	160	156
Seropositivity rate at day 365 (95% Confidence Interval)		
Day 365, TDV-1	84.4 (77.8 to 89.6)	89.7 (83.9 to 94.0)
Day 365, TDV-2	98.8 (95.6 to 99.8)	91.0 (85.4 to 95.0)
Day 365, TDV-3	79.4 (72.3 to 85.4)	77.6 (70.2 to 83.8)
Day 365, TDV-4	72.5 (64.9 to 79.3)	81.4 (74.4 to 87.2)

b) Geometric Mean Neutralizing Antibody Titers (GMTs)

Neutralizing antibody titers (GMTs) for each dengue serotype were determined in a serum sample of a subject taken before administration of the first unit dose of the dengue vaccine composition and 180 or 365 days after administration of the first unit dose of the dengue vaccine composition using the microneutralization (MNT) assay as described in Example 2.

For both HD-TDV and TDV, an increase in GMTs was observed by Day 15, reaching a maximum by Day 30 (FIG. 4). Antibody titers remained above baseline levels throughout the trial for both unit doses. In the overall trial population, no substantial differences were seen in GMT titers between the two unit dose groups, except against DENV-2, where the response was higher for the HD-TDV group compared with the TDV group (8640.3 versus 1992.7 at Day 30). When assessed by baseline seropositivity status, the GMT profiles were similar as for the entire population, with a rise by Day 15, peak at Day 30, and gradual decline thereafter (FIG. 4B). In the group who were seropositive at baseline, the difference between the unit dose groups in response to DENV-2 was reduced, with GMTs of 6970.3 and 4193.3 at Day 30 for the HD-TDV and TDV groups, respectively. Responses were higher against DENV-1,

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DENV-3, and DENV-4 in the baseline seropositive group, compared with the baseline seronegative group, across both unit doses. Against DENV-2, a lower response was seen in baseline seronegative subjects receiving TDV, compared with HD-TDV; Day 30 GMTs were 812.9 in the TDV group,

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compared with 10965.9 in the HD-TDV group. The response in these subjects also differed against DENV-4, with a higher response being observed in the TDV group (Day 30 GMTs of 57.7, compared with 20.9 in the HD-TDV group); this difference persisted to Day 365 (FIG. 4A).

TABLE 9

Geometric mean neutralizing antibody titers (GMTs) at day 180		
	Comparative unit dose	Example 1 unit dose
Overall number of participants analyzed	166	163
GMTs (95% Confidence Interval) [units: Titer]		
Day 180, TDV-1	379.4 (252.3 to 570.3)	312.2 (212.2 to 459.2)
Day 180, TDV-2	2585.5 (2088.8 to 3200.3)	1235.0 (890.7 to 1712.5)
Day 180, TDV-3	236.2 (162.2 to 344.0)	161.0 (110.5 to 234.6)
Day 180, TDV-4	91.0 (65.7 to 125.9)	92.9 (68.9 to 125.4)

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TABLE 10

Ratio of geometric mean neutralizing antibody titers (GMTs) at day 180		
	Comparative unit dose	Example 1 unit dose
TDV-2:TDV-1	7	4
TDV-2:TDV-3	11	8
TDV-2:TDV-4	28	13

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TABLE 11

Geometric mean neutralizing antibody titers (GMTs) at day 365		
	Comparative unit dose	Example 1 unit dose
Overall number of participants analyzed	160	156
GMTs (95% Confidence Interval) [units: Titer]		
Day 365, TDV-1	247.3 (160.9 to 380.2)	264.1 (181.1 to 385.1)
Day 365, TDV-2	1726.0 (1392.6 to 2139.3)	809.5 (576.6 to 1136.4)
Day 365, TDV-3	163.2 (110.0 to 242.3)	132.6 (89.9 to 195.5)
Day 365, TDV-4	75.3 (53.8 to 105.4)	77.0 (56.9 to 104.2)

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TABLE 12

Ratio of geometric mean neutralizing antibody titers (GMTs) at day 365		
	Comparative unit dose	Example 1 unit dose
TDV-2:TDV-1	7	3
TDV-2:TDV-3	11	6
TDV-2:TDV-4	23	11

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TABLE 13

Geometric mean neutralizing antibody titers (GMTs) of all four dengue serotypes assessed by dengue baseline seropositivity status at day 180		
	Comparative unit dose	Example 1 unit dose
Seropositive	89 Participants	88 Participants
Day 180, TDV-1 (Seropositive)	2327.2 (1550.4 to 3493.3)	1356.2 (905.5 to 2031.2)
Day 180, TDV-2 (Seropositive)	4412.0 (3586.6 to 5427.4)	2952.0 (2358.2 to 3695.4)

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TABLE 13-continued

Geometric mean neutralizing antibody titers (GMTs) of all four dengue serotypes assessed by dengue baseline seropositivity status at day 180		
	Comparative unit dose	Example 1 unit dose
Day 180, TDV-3 (Seropositive)	1248.3 (879.7 to 1771.3)	693.6 (459.6 to 1046.6)
Day 180, TDV-4 (Seropositive)	399.5 (291.3 to 547.9)	268.3 (190.2 to 378.6)
Seronegative	77 Participants	75 Participants
Day 180, TDV-1 (Seronegative)	46.6 (32.0 to 67.9)	55.7 (35.6 to 87.1)
Day 180, TDV-2 (Seronegative)	1394.1 (983.2 to 1976.6)	444.3 (247.2 to 798.5)
Day 180, TDV-3 (Seronegative)	34.5 (23.4 to 50.7)	29.0 (19.4 to 43.3)
Day 180, TDV-4 (Seronegative)	16.4 (12.3 to 22.0)	26.8 (19.0 to 37.7)

TABLE 14

Ratio of geometric mean neutralizing antibody titers (GMTs) assessed by dengue baseline seropositivity status at day 180 and 365		
	Comparative unit dose	Example 1 unit dose
Seropositive 180 Days		
TDV-2:TDV-1	1.9	2.2
TDV-2:TDV-3	3.5	4.3
TDV-2:TDV-4	11.0	11.0
Seronegative 180 Days		
TDV-2:TDV-1	29.9	8.0
TDV-2:TDV-3	40.4	15.3
TDV-2:TDV-4	85.0	16.6

TABLE 15

Geometric mean neutralizing antibody titers (GMTs) of all four dengue serotypes assessed by dengue baseline seropositivity status at day 365		
	Comparative unit dose	Example 1 unit dose
Seropositive	84 Participants	85 Participants
Day 365, TDV-1 (Seropositive)	1633.3 (1055.8 to 2526.7)	1081.5 (724.0 to 1615.6)
Day 365, TDV-2 (Seropositive)	3316.0 (2623.8 to 4190.9)	2177.3 (1613.5 to 2938.1)
Day 365, TDV-3 (Seropositive)	830.6 (551.2 to 1251.5)	600.2 (402.3 to 895.3)
Day 365, TDV-4 (Seropositive)	346.3 (249.2 to 481.1)	212.6 (152.2 to 296.9)
Seronegative	76 Participants	71 Participants
Day 365, TDV-1 (Seronegative)	30.7 (20.4 to 46.2)	48.8 (32.1 to 74.2)
Day 365, TDV-2 (Seronegative)	838.7 (621.9 to 1131.1)	247.6 (143.9 to 426.1)
Day 365, TDV-3 (Seronegative)	27.0 (17.8 to 41.1)	21.7 (14.3 to 33.1)
Day 365, TDV-4 (Seronegative)	13.9 (10.3 to 19.0)	22.9 (15.8 to 33.1)

TABLE 16

Ratio of geometric mean neutralizing antibody titers (GMTs) assessed by dengue baseline seropositivity status at day 365		
	Comparative unit dose	Example 1 unit dose
Seropositive 365 Days		
TDV-2:TDV-1	2.0	2.0
TDV-2:TDV-3	4.0	3.6
TDV-2:TDV-4	9.6	10.2
Seronegative 365 Days		
TDV-2:TDV-1	27.3	5.1
TDV-2:TDV-3	31.1	11.4
TDV-2:TDV-4	60.3	10.8

c) Safety

Overall, rates of solicited local and systemic adverse events (AEs), unsolicited AEs, and serious adverse events (SAEs) were similar across the two unit dose groups. No

deaths or AEs leading to discontinuation were recorded in the trial. Three subjects in each unit dose group experienced SAEs, one of these events in the HD-TDV group was considered by the sponsor to be vaccine-related based on temporal association. The SAE was polyarthritis which began six days following receipt of the vaccine. Rates of solicited reactions were similar across unit dose groups, and seropositivity status at baseline. Overall, 47.4% and 53.7% of subjects reported local reactions, and 52.0% and 50.9% reported solicited systemic AEs, in the HD-TDV and TDV groups, respectively. Most reactions were mild or moderate. The most commonly reported local adverse reaction was injection site pain (46.3% in the HD-TDV group, 52.0% in the TDV group) and the most common systemic AE was headache (28.6% in the HD-TDV group, 34.9% in the TDV group).

d) Conclusion

Both unit doses showed an acceptable safety profile. The results show a more balanced immune response with the new TDV unit dose compared to the early HD-TDV unit dose, particularly in the subjects who were seronegative prior to vaccination: (i) in baseline seronegative subjects, response to DENV-2 was less dominant with TDV and (ii) DENV-4 seropositivity rates and GMTs were also higher with TDV in these subjects.

Example 4

60 Cell-Mediated Immunity Stimulated by the Dengue Vaccine

A gamma interferon ($\text{IFN}\gamma$) enzyme-linked immunosorbent spot (ELISPOT) assay was performed using peripheral blood mononuclear cells (PBMCs) from the subjects taking part in the clinical trial of Example 3 and the commercial ELISpot assay kit available from Mabtech according to the

manufacturer's instructions. Briefly, cryopreserved PBMCs were thawed and left to rest overnight, then incubated with various peptide pools for 18-22 hours in plates coated with anti-IFN γ antibodies. PBMCs were then removed and spots were developed and then counted. Results were reported as mean spot forming cells (SFC) per 10^6 PBMCs. Peptide pools matched selected DENV-derived proteins, covering the entire DENV-2 proteome with NS1, NS3, and NS5 proteins from New Guinea C (NGC) and Thailand/16681/84 strains; and C, prM+E, NS2 and NS4 proteins from Thailand/16681/84 only plus TDV-1, TDV-3 and TDV-4 prM+E inserts from DENV-1, -3 and -4 strains Thailand/16007/1964, Philippines/16562/1964 and Indonesia/1036/1976, respectively.

Response rates to DENV-2 proteome at 6 and 12 months post-single dose of TDV were >90%, and were comparable between subjects seronegative and seropositive at baseline (FIG. 5).

MEC) as measured by transendothelial electrical resistance (TEER) (Puerta-Guardo et al. (2016) PLoS Pathog. 12(7): e1005738). It also interacts with endothelial cells to induce degradation of the glycocalyx via activation of sialidases and the cathepsin L/heparanase pathway (Glasner et al. (2017) PLoS Pathog. 13(11): e1006673). In view of these effects, it was investigated whether the comparative unit dose induces antibodies against NS1 and inhibits NS1-mediated physiological effects.

a) Anti-NS1 Antibodies

Serum samples were collected at day 0 before vaccination and day 120 after administration of the first unit dose. Serum was collected from 6 dengue seronegative and 6 dengue seropositive subjects at both day 0 and day 120, and Ab concentrations were determined by ELISA.

The anti-NS1 antibody concentration in seronegative and seropositive subjects at day 0 and day 120 is shown in Tables 17 and 18:

TABLE 17

Anti-NS1 antibody concentration in seronegative subjects at day 0 and day 120								
Dengue seronegative subjects								
Anti-NS1 antibody concentration (RU/ml)								
Subj.	DENV1	DENV2	DENV3	DENV4	Subj.	DENV1	DENV2	DENV4
	d0	d120	d0	d120		d0	d120	d0
1023014	13.49	602.56	16.22	2570.40	10.00	489.78	28.18	302.00
1025011	66.07	173.78	35.48	794.33	67.61	117.49	42.66	85.11
1025013	5.62	380.19	24.55	2454.71	16.98	316.23	10.00	186.21
1035002	34.67	177.83	31.62	977.24	17.78	114.82	19.05	44.67
1035005	50.12	467.74	20.42	1659.59	104.71	309.03	66.07	288.40
1035001	40.74	186.21	52.48	489.78	44.67	169.82	51.29	177.83

TABLE 18

Anti-NS1 antibody concentration in seronegative subjects at day 0 and day 120								
Dengue seropositive subjects								
Anti-NS1 antibody concentration (RU/ml)								
Subj.	DENV1	DENV2	DENV3	DENV4	Subj.	DENV1	DENV2	DENV4
	d0	d120	d0	d120		d0	d120	d0
1052010	691.83	11481.54	309.03	12022.64	436.52	7585.78	245.47	4677.35
1052014	758.58	1445.44	407.38	891.25	758.58	1122.02	724.44	707.95
1052015	3890.45	3467.37	2570.40	2344.23	3235.94	2818.38	660.69	707.95
1071007	478.63	851.14	239.88	478.63	660.69	1202.26	870.96	1258.93
1071012	691.83	776.25	724.44	676.08	776.25	812.83	346.74	446.68
1082009	5888.44	5370.32	7413.10	6309.57	5248.07	4897.79	891.25	794.33

The response was primarily directed to the NS proteins, particularly in subjects seronegative at baseline (FIG. 6).

The NS3 and NS5 proteins were the most recognized antigens (by 50-75% of subjects). Immunodominance of NS3 and NS5 was independent of baseline serostatus. Durability of the response was maintained equally between NS3 and NS5 throughout the 12-month post-single vaccination follow-up.

Example 5

Antibody Responses to Non-Structural Proteins

The non-structural protein NS-1 from all four dengue serotypes can induce endothelial hyperpermeability of human pulmonary microvascular endothelial cells (HP-

These data show that the vaccine induces antibodies against NS1 from all dengue serotypes in both seropositive and seronegative subjects.

b) Transendothelial Electrical Resistance (TEER)

The effect of recombinant NS1 proteins from dengue serotypes 1, 2, 3 and 4 and sera from vaccinated seronegative and seropositive subjects on endothelial permeability was evaluated by measuring TEER of HPMEC grown on a 24-well Transwell polycarbonate membrane system (Transwell® permeable support, 0.4 μ M, 6.5 mm insert; Corning Inc.) as previously described (Beatty et al. (2015) Sci. Transl. Med. 7(304): 304ra141; Puerta-Guardo et al. (2016) PLoS Pathog. 12(7): e1005738). Briefly, TEER was measured in Ohms (Ω) at sequential 2-hour time-points following the addition of test proteins using an Epithelial Volt Ohm Meter (EVOM) with "chopstick" electrodes (World Precision

Instruments). Untreated endothelial cells grown on Transwell inserts were used as negative untreated controls, and inserts with medium alone were used for blank resistance measurements. Relative TEER represents a ratio of resistance values (Ω) as follows: $(\Omega_{\text{experimental condition}} - \Omega_{\text{medium alone}}) / (\Omega_{\text{non-treated endothelial cells}} - \Omega_{\text{medium alone}})$. After 24 hours of treatment, 50% of upper and lower chamber media was replaced by fresh endothelial cell medium. For experiments using sera, 30 μl of culture supernatant was removed from the apical chamber and replaced with 30 μl of serum samples immediately before the addition of 5 $\mu\text{g/ml}$ DENV-2 NS1.

Day 0 serum samples from seronegative subjects did not protect against NS1-mediated barrier dysfunction, but day 120 samples from all seronegative subjects blocked decreases in TEER values induced by NS1 (see FIG. 7A). Day 0 samples from seropositive subjects produced varying levels of protection, and all day 120 samples from seropositive subjects completely abrogated NS1-induced hyperpermeability (see FIG. 7B).

c) Degradation of Glycocalyx-Like Layer (EGL)

Microscopy was performed as previously described (Puerta-Guardo et al. (2016) PLoS Pathog. 12(7):e1005738). For imaging experiments, HPMEC were grown on cover-slips coated with 0.2% gelatin (Sigma) and imaged on a Zeiss LSM 710 Axio Observer inverted fluorescence microscope equipped with a 34-channel spectral detector. Images acquired using the Zen 2010 software (Zeiss) were processed and analyzed with ImageJ software. All RGB images were converted to grayscale, then mean grayscale values and integrated density from selected areas were taken, along with adjacent background readings, and plotted as mean fluorescence intensity (MFI). To assess the effect of sera from vaccinated subjects on DENV2 NS1-induced EGL disruption, the distribution of sialic acid and heparan sulfate was examined on confluent HPMEC monolayers treated with DENV2 NS1 (5 $\mu\text{g/ml}$)+negative control serum (30 μl), NS1+positive control serum (30 μl), or NS1+serum from vaccinated subjects (30 μl) and fixed with 4% paraformaldehyde (PFA) at 6 hours post-treatment. Primary antibodies (Wheat germ agglutinin (WGA) lectin conjugated to Alexa Fluor 647 (WGA-A647, Molecular Probes) to stain N-acetyl neuraminic acid (sialic acid); Ab Heparan Sulfate, purified (clone F58-10E4, Amsbio) were incubated overnight at 4° C., and detection was performed using secondary species-specific anti-IgG or anti-IgM antibodies conjugated to Alexa fluorophores (488 and 647).

Day 0 sera from seronegative subjects had no substantial protective effect, while day 120 sera from seronegative subjects completely blocked degradation of both sialic acid and heparan sulfate. Similarly, day 0 samples from seropositive subjects exhibited varying levels of protection, and sera from seropositive subjects at day 120 were completely protective (see FIG. 8). Positive control serum was used as a baseline for protection, and negative control serum represented maximum NS1-mediated disruption. These results show that the anti-NS1 antibody response stimulated by the dengue vaccine can protect against NS1-induced hyperpermeability by preventing the degradation of key EGL components.

Taken together, these results suggest that the dengue vaccine stimulates robust and protective anti-DENV2 NS1 Ab responses following vaccination.

Example 6

Phase III Clinical Trial in Children

A Phase III, double-blind, randomized, and placebo-controlled trial in 20100 subjects aged 4 to 16 years living

in Thailand, Sri Lanka, Philippines, Panama, Nicaragua, Dominican Republic, Colombia or Brazil was performed evaluating the efficacy, safety and immunogenicity of a tetravalent dengue vaccine referred to hereinafter as TDV.

5 The trial includes 3 parts. Part 1 evaluates vaccine efficacy (VE) and lasts until both of the following 2 criteria are fulfilled: (i) 120 cases of dengue fever are confirmed and (ii) minimum duration of subject follow-up of 12 months post-second vaccination. Part 2 is for an additional 6 months to 10 evaluate VE and for secondary efficacy analyses. Part 3 will evaluate long-term safety by following participants for side effects and will last an additional 3 years.

Part 1: Active surveillance for the primary assessment of 15 efficacy in all subjects. During this time subjects were contacted at least weekly to ensure identification of febrile illness that could potentially be due to dengue. This part commenced on the day of vaccination and finished once both of the following 2 criteria were fulfilled: (i) 120 cases of dengue fever are confirmed and (ii) minimum duration of 20 subject follow-up of 12 months post-second vaccination. The end of Part 1 was defined for each subject so that the duration of follow up after the second vaccination was approximately the same for all subjects. Virologically-confirmed cases in Part 1 count towards the primary efficacy objective if occurring at least 30 days post-second vaccination.

Part 2: Active surveillance for an additional 6 months for 25 each subject following the completion of Part 1. During this time subjects were contacted at least weekly to ensure identification of febrile illness that could potentially be due to dengue. Virologically-confirmed cases in Parts 1 and 2 contribute towards the secondary efficacy objectives.

Part 3: Modified active surveillance for the assessment of 30 safety in all subjects following the completion of Part 2 and lasting 3 years for each subject. The modified surveillance during Part 3 will maintain at least weekly contacts through Part 3 of the trial, but the intensity of investigation will be modified based on the need for hospitalization. Surveillance will identify febrile illness of any severity that could potentially be due to dengue.

Criteria for Inclusion include:

The subject was aged 4 to 16 years inclusive, at the time of randomization.

Individuals who were 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.

The subject and/or the subject's parent/guardian signed and dated an assent/written informed consent form where applicable, and any required privacy authorization prior to the initiation of any trial procedures, after the nature of the trial has been explained according to local regulatory requirements.

Individuals who can comply with trial procedures and are available for the duration of follow-up.

Exclusion criteria include:

1. Febrile illness (temperature 38° C.) or moderate or severe acute illness or infection at the time of randomization.

2. History or any illness that, in the opinion of the Investigator, might interfere with the results of the trial or pose an additional risk to the subject due to participation in the trial, including but not limited to:

a. Known hypersensitivity or allergy to any of the vaccine components.

b. Female subjects (post-menarche) who are pregnant or breastfeeding.

- c. Individuals with any serious chronic or progressive disease according to judgment of the Investigator (eg, neoplasm, insulin-dependent diabetes, cardiac, renal or hepatic disease, neurologic or seizure disorder or Guillain-Barré syndrome).
- d. Known or suspected impairment/alteration of immune function, including:
 - i. Chronic use of oral steroids (equivalent to 20 mg/day prednisone weeks/mg/kg body weight/day prednisone weeks) within 60 days prior to Day 1 (Month 0) (use of inhaled, intranasal, or topical corticosteroids is allowed).
 - ii. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone weeks/mg/kg body weight/day prednisone weeks) within 60 days prior to Day 1 (Month 0).
 - iii. Administration of immunoglobulins and/or any blood products within the 3 months prior to Day 1 (Month 0) or planned administration during the trial.
 - iv. Receipt of immunostimulants within 60 days prior to Day 1 (Month 0).
 - v. Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (Month 0).
 - vi. Human Immunodeficiency Virus (HIV) infection or HIV-related disease.
 - vii. Genetic immunodeficiency.
- 3. Receipt of any other vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 (Month 0) or planning to receive any vaccine within 28 days after Day 1 (Month 0).
- 4. Participation in any clinical trial with another investigational product 30 days prior to Day 1 (Month 0) or intent to participate in another clinical trial at any time during the conduct of this trial.
- 5. Previous participation in any clinical trial of a dengue candidate vaccine, or previous receipt of a dengue vaccine.
- 6. First degree relatives of individuals involved in trial conduct.
- 7. Females of childbearing potential who are sexually active, and who have not used any of the acceptable contraceptive method for at least 2 months prior to Day 1 (Month 0).
- 8. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method up to 6 weeks post-second vaccination.
- 9. Deprived of freedom by administrative or court order, or in an emergency setting, or hospitalized involuntarily.
- 10. Current alcohol abuse or drug addiction that may interfere with the subject's ability to comply with trial procedures.
- 11. Identified as an employee of the Investigator or trial center, with direct involvement in the proposed trial or other trials under the direction of that Investigator or trial center.

Eligible subjects were randomized (2:1) into two treatment groups: group 1 received one subcutaneous (SC) dose of TDV in the upper arm on Day 1 and on Day 90, and group 2 received one subcutaneous dose of placebo in the upper arm on Day 1 and on Day 90. Randomization was stratified by region (Asia Pacific and Latin America) and age range (children aged 4-5 years, 6-11 years, and 12-16 years) to ensure each age range has the appropriate ratio of TDV to placebo in each region. After randomization dropouts were

not replaced. Study Day 1 is defined to be the date of the first dose administration of TDV or placebo. The TDV was prepared as described in Example 1. Each subcutaneous dose of TDV was 0.5 mL and the concentration of the four dengue serotypes in the TDV vaccine in each dose was 3.6 log₁₀ PFU/dose, 4.0 log₁₀ PFU/dose, 4.6 log₁₀ PFU/dose and 5.1 log₁₀ PFU/dose of TDV-1, TDV-2, TDV-3 and TDV-4, respectively.

Primary Outcome Measures included the vaccine efficacy (VE) of two doses of TDV in preventing virologically-confirmed dengue (VCD) fever induced by any dengue serotype [time frame: 30 days post-second vaccination (Day 120) until the end of Part 1]. VE is defined as $1 - (\lambda_v/\lambda_c)$, wherein λ_v and λ_c denote the hazard rates for the TDV and placebo groups, respectively. A virologically-confirmed dengue case is defined as febrile illness (defined as temperature $\geq 38^\circ\text{C}$. on any 2 of 3 consecutive days) or illness clinically suspected to be dengue by the Investigator with a positive serotype-specific reverse transcriptase polymerase chain reaction (RT-PCR). A febrile illness will require an interval of at least 14 days from a previous febrile illness to avoid overlap of acute and convalescent visits from one episode with those from a second episode.

Secondary Outcome Measures include:

- 1) VE of two doses of TDV in preventing virologically-confirmed dengue fever induced by each dengue serotype [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2].
- 2) VE of two doses of TDV in preventing virologically-confirmed dengue fever induced by any dengue serotype in participants dengue seronegative at baseline [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2 (up to 21 months)].
- 3) VE of two doses of TDV in preventing virologically-confirmed dengue fever induced by any dengue serotype in participants dengue seropositive at baseline [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2].
- 4) VE of two doses of TDV in preventing hospitalization due to virologically-confirmed dengue fever induced by any dengue serotype [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2].
- 5) VE of two doses of TDV in preventing virologically-confirmed severe dengue fever induced by any dengue serotype [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2].
- 6) Percentage of participants with solicited local injection site adverse events (AEs) in the safety subset [time frame: Days 1 through 7 after each vaccination] and severity of solicited local injection AEs. Solicited local AEs at injection site are defined as pain, erythema and swelling that occurred within 7 days after each vaccination.
- 7) Percentage of participants with solicited systemic adverse events (AEs) in the safety subset [time frame: Days 1 through 14 after each vaccination] and severity of solicited systemic AEs. Solicited systemic AEs in children (<6 years) are defined as fever, irritability/fussiness, drowsiness and loss of appetite that occurred within 14 days after each vaccination. Solicited systemic AEs in children (6 years) are defined as fever, headache, asthenia, malaise and myalgia that occurred within 14 days after each vaccination.
- 8) Percentage of participants with any unsolicited adverse events (AEs) in the safety subset [time frame: Days 1

through 28 after each vaccination]. Unsolicited AEs are any AEs that are not solicited local or systemic AEs, as defined above.

- 9) Percentage of participants with serious adverse events (SAEs) during Parts 1 and 2 [time frame: from Day 1 until the end of Parts 1 and 2]. A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect or is medically important due to other reasons than the above mentioned criteria.
- 10) Percentage of participants with fatal SAEs and SAEs related to study drug during the first and second half of Part 3 [time frame: for 3 years (18 month halves) beginning at the end of Part 2 (approximately 21 months after the first vaccination)].
- 11) Percentage of participants with a seropositive response for each of the four dengue serotypes in the immunogenicity subset [time frame: Day 1 and months 1, 3, 4, 9, 15 and then annually (up to 3 years)]. Seropositive response is defined as a reciprocal neutralizing titer ≥ 10 . The four DENV serotypes are DEN-1, DEN-2, DEN-3 and DEN-4.
- 12) Percentage of participants with a seropositive response for multiple dengue serotypes in the immunogenicity subset [time frame: Day 1 and months 1, 3, 4, 9, 15 and then annually (up to 3 years)].
- 13) Geometric Mean Titers (GMTs) of neutralizing antibodies for each of the four dengue serotypes in the immunogenicity subset [time frame: Day 1 and months 1, 3, 4, 9, 15 and then annually (up to 3 years)]. GMTs of neutralizing antibodies will be measured via microneutralization test (MNT) as described in Example 2.

a) Study Population

After screening, 20,099 participants were randomized, and 20,071 received at least one injection. In total, 97.4% of placebo participants (n/N: 6,521/6,698) and 97.3% of vaccinees (n/N: 13,038/13,401) completed Part 1 of the study (FIG. 9). Reasons for study withdrawals included AEs, participants lost to follow-up, pregnancy, protocol violations, and withdrawal by participants (or parents/guardians). Baseline characteristics were similar across both treatment groups (Table 19). Mean age of study participants was 9.6 years, with baseline seronegativity of 27.7%, and enrollment was broadly balanced across regions (46.5% in Asia, 53.5% in Latin America). The highest seronegative rate was in Panama (62.2%), followed by Sri Lanka (38.5%), Thailand (34.4%), Brazil (28.8%), Nicaragua (22.3%), Colombia (15.4%), the Philippines (12.4%), and the Dominican Republic (2.8%).

TABLE 19

Baseline characteristics of study population (number, %)			
	TDV	Placebo	Total
Per Protocol Set			
Number of Participants	12,704	6,317	19,021
Mean Age (Years, SD)	9.6 (3.35)	9.6 (3.34)	9.6 (3.35)
Baseline Seronegative ^a	3,533 (27.8)	1,726 (27.3)	5,259 (27.7)

TABLE 19-continued

Baseline characteristics of study population (number, %)			
	TDV	Placebo	Total
Female	6,314 (49.7)	3,098 (49.0)	9,412 (49.5)
Male	6,390 (50.3)	3,219 (51.0)	9,609 (50.5)
Asia Pacific	5,896 (46.4)	2,942 (46.6)	8,838 (46.5)
Baseline	1,503 (25.5)	773 (26.3)	2,276 (25.8)
Seronegative ^a			
Latin America	6,808 (53.6)	3,375 (53.4)	10,183 (53.5)
Baseline	2,030 (29.8)	953 (28.2)	2,983 (29.3)
Seronegative ^a			
Safety Set ^b			
Number of Participants	13,380	6,687	20,071
Mean Age (Years, SD)	9.6 (3.36)	9.6 (3.34)	9.6 (3.35)
Baseline	3,714 (27.8)	1,832 (27.4)	5,547 (27.6)
Seronegative ^a			
Female	6,651 (49.7)	3,276 (49.0)	9,929 (49.5)
Male	6,729 (50.3)	3,411 (51.0)	10,142 (50.5)
Safety Set of Subset ^b			
Number of Participants	2,663	1,329	3,993
Baseline	740 (27.8)	369 (27.8)	1,109 (27.8)
Seronegative ^a			

^aSeronegative for all serotypes; seropositive defined as reciprocal neutralizing antibody titer ≥ 10 ; SD, standard deviation.

^bnumbers of participants in TVD plus placebo groups are not equal to total numbers shown because misallocated participants (i.e. those who received both TVD and placebo due to an administrative error) are not included in the TVD and placebo group data.

b) Febrile Illnesses and VCD

During Part 1, 5,754 and 4,663 episodes of febrile illness were reported in Asian and Latin American sites, respectively. Acute samples were obtained in 99.5% and 96.6% of these cases, with 98.3% and 85.1% of samples taken within five days, in Asia and Latin America, respectively. There were 278 VCD cases (76 hospitalized) in the safety set during the entire Part 1 period, of which 210 (58 hospitalized) were 30 days post-second vaccination in the PPS (Table 20; Table 22) and were included in primary endpoint analysis.

c) Distribution of VCD Included in Primary Endpoint Analysis

DENV-1 was reported in all countries with VCD and included all the 21 cases in Panama. In Sri Lanka, 54 of 60 VCD were DENV-2, and 87 of 109 VCD in the Philippines were DENV-3. All seven DENV-4 VCD were reported in the Philippines. No VCD were reported in Nicaragua or the Dominican Republic. Of the associated 58 hospitalized VCD, 43 were reported in Sri Lanka. A total of two severe dengue (both DENV-3) and five dengue hemorrhagic fever (DHF; three DENV-2; two DENV-3) cases were reported (Table 21). These seven were also the only such cases in the entire part 1 safety set.

d) Vaccine Efficacy

VE against VCD of any serotype was 80.2% (95% CI: 73.3-85.3; P<0.001). A similar efficacy of 81% (95% CI: 64.1-90.0) between the doses and from first dose onwards in the safety set (Table 20) suggests that the vaccine was efficacious after the first dose. Exploratory analysis of the secondary efficacy endpoints showed a trend of differential efficacy by serotype, with the highest efficacy against DENV-2 (97.7%), followed by DENV-1 (73.7%), DENV-4 (63.2% with CI containing zero), and DENV-3 (62.6%; Table 3). Overall, efficacy was similar in baseline seronegatives (74.9%) and seropositives (82.2%; FIG. 10A); however, this varied by serotype. Efficacy against DENV-2 was not impacted by serostatus; efficacy against DENV-1 was

slightly higher in baseline seropositives (79.8%; 95% CI: 51.3-91.6) than baseline seronegatives (67.2%; 95% CI: 23.2-86.0). No efficacy was observed against DENV-3 in baseline seronegatives (-38.7%; 95% CI: -335.7-55.8) compared to baseline seropositives (71.3%; 95% CI: 54.2-82.0). Efficacy by serostatus could not be calculated for DENV-4 because no cases were observed in baseline seronegatives. In the primary endpoint timeframe of the PPS, only five VCD requiring hospitalization were reported in the vaccine group compared with 53 cases in the placebo group, with a VE of 95.4% (95% CI: 88.4-98.2; 97.2% for baseline seronegatives and 94.4% for baseline seropositives; Table 21; FIG. 10B), consistent with a VE of 93.3% (95% CI: 86.7-96.7) in the safety set from first dose onwards.

The primary vaccine efficacy (VE) of two doses of TDV in preventing virologically-confirmed dengue (VCD) fever induced by any dengue serotype is shown in Table 20.

TABLE 20

Vaccine efficacy of TDV in preventing virologically confirmed dengue (VCD) fever against any serotype from 30 days post-second vaccination until end of part 1 Per Protocol Set (PPS). Safety set analysis from first dose to end of Part 1 study period		
	Placebo n = 6317	TDV (PPS) n = 12,704
number of subject evaluated	6,316	12,700
number of subjects with febrile illness	1,712	3,195
number of febrile illness cases	2,591	4,692
virologically confirmed dengue fever (n [%])	149 [2.4]	61 [0.5]
Person-years at risk	5,670.1	11,578.7
incident density	2.6	0.5
relative risk	0.20	
95% CI of relative risk	(0.15, 0.27)	
vaccine efficacy (%)	80.2	
95% CI of vaccine efficacy	(73.3, 85.3)	
p-value for vaccine efficacy	<0.001	
	Placebo	TDV (Safety Set)*
number of subject evaluated	6,687	13,380
virologically confirmed dengue fever (n [%])	199 [3.0]	78 [0.6]
Person-years at risk	8,072.0	16,351.5
incident density	2.5	0.5
vaccine efficacy (%)	80.9	
95% CI of vaccine efficacy	(75.2, 85.3)	

Note 1:

Percentage of virologically confirmed dengue (VCD) fever are based on number of subjects evaluated.

Note 2:

Person-years at risks is defined as cumulative time in years until start of VCD fever or until end of Part 1 study period or discontinuation date, whichever comes first. Incident density is defined as the number of cases per 100 person-years at risk. Percentages are based on total number (denominator) of analysis set participants evaluated and may not be equal to the total number of participants in the per protocol analysis set.

*One participant had two instances of VCD during Part 1, only the first VCD was included in efficacy calculation

Note 3:

Vaccine efficacy (VE) and 2-sided 95% CIs are estimated from a Cox proportional hazard model with TDV as a factor, adjusted for age and stratified by region.

Note 4:

Statistical significance will be concluded if the lower bound of the 95% CI for VE is above 25%. Since the hypotheses will be tested in a confirmatory manner at a 2-sided significance level of 5%, the calculated p-value should be compared with 0.025.

Note 5:

Relative risk is calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group.

For the efficacy evaluation shown in Table 20, a case of VCD was defined as febrile illness (defined as fever 38° C. on any 2 of 3 consecutive days) with a positive serotype-specific RT-PCR (i.e., positive dengue detection RT-PCR) and occurring at any time starting from 30 days post-second

vaccination (Day 120 [Month 4]) through the end of Part 1. The analysis was performed on the Per-Protocol Set (PPS) and Safety Set.

As used herein, the “Per-Protocol Set (PPS)” consist of all subjects in the Full Analysis Set (FAS) consisting of all randomized subjects who received at least one dose of TDV or placebo who had no major protocol violations. Major protocol violations are not receiving both doses of TDV or placebo administration, not receiving both doses in the correct interval, not having the correct administration of TDV or placebo, use of prohibited medications/vaccines by the subject, the subject meets any of the exclusion criteria of 2d, 3, 4 or 5 defined above or product preparation error.

The p-value is obtained by solving the critical value Z in the following equation:

$$\text{Upper bound of 1-sided } (1-p\%) \text{ CI of HR} = 0.75, \\ \text{wherein HR is the hazard ratio and defined as} \\ \text{HR} = \lambda V / \lambda C.$$

$$e^{[\hat{\beta} + Z^* S'E]} = 0.75, \text{ wherein } \hat{\beta} \text{ defines the treatment} \\ \text{and } S'E \text{ the related standard error.}$$

The 1-sided p-value is 1-(area to the left of the critical value Z from a standard normal distribution). Since the hypotheses will be tested in a confirmatory manner 2-sided at a significance level of 5%, the calculated 1-sided p-value should be compared with 0.025.

In summary in Part 1 of this study, a high vaccine efficacy of 80.2% against virologically-confirmed dengue of any serotype in children 4-16 years of age was found. It included an efficacy of 74.9% in baseline seronegatives and a robust 95.4% reduction in hospitalizations. Onset of protection could be seen after the first dose with 81% efficacy between doses. Overall, these results suggest a potential benefit for each vaccine recipient regardless of prior dengue exposure or age. This finding is significant because vaccine development against dengue has been challenging, especially for dengue naïve individuals, and dengue remains one of the WHO's top ten threats to global health in 2019.¹⁹ Furthermore, the onset of protection after the first dose has potential utility in the context of outbreak control or travel vaccination, offering a reduction in the risk of dengue after only one dose.

Severe forms of dengue were assessed as follows: Dengue Hemorrhagic Fever (DHF) as defined by the 1997 WHO definition. Severe Dengue through the Dengue Case Adjudication Committee. The Dengue Case Adjudication Committee (DCAC) consisted of four members: a voting chairperson, two voting members, and an independent non-voting statistician. The three DCAC voting members are all physicians and clinical dengue experts. DCAC members are not study investigators and do not have any conflict of interest that would bias their review of the trial data. All non-hospitalized cases were considered non-severe. The DCAC severe dengue case criteria applied in a blinded manner to virologically-confirmed hospitalized dengue cases are as follows: 1) bleeding abnormality, for a case to be considered severe there needs to be a significant intervention required in response to the bleeding episode such as blood transfusion, nasal packing, hormonal therapy, or, bleeding occurred into critical organs such as the brain; 2) plasma leakage, for a case to be considered severe there needs to be evidence of both plasma leakage and functional impairment (plasma leakage includes clinical evidence, radiological evidence, or hematocrit elevated >20% above normal levels or baseline; functional impairment defined as shock or respiratory distress); 3) liver, for a case to be considered severe there needs to be evidence of both hepatitis and functional impairment

(hepatitis defined as an aspartate aminotransferase [AST] or alanine aminotransferase [ALT]>10 upper limit of normal range [ULN]; functional impairment defined as prothrombin [PT]>1.5 ULN or hypoalbuminemia); 4) renal, serum creatinine >2.5 times ULN or requiring dialysis; 5) cardiac, abnormalities intrinsic to the heart (i.e. not resulting from intravascular volume depletion) and with evidence of functional impairment (examples of intrinsic abnormality: myocarditis, pericarditis, and myopericarditis; example of func-

tional impairment: new conduction abnormality resulting in irregular heart rhythm [i.e. not transient first-degree heart block]); 6) central nervous system, any abnormality with the exception of a simple febrile convulsion or a brief delirium; 7) shock, all shock cases considered severe. At least 1 functional impairment (of criterion 3,4,5,6), needs to be present but the totality of data were considered by the members in their assessment.

TABLE 21

Distribution of cases contributing to primary endpoint by per protocol set subgroup					
	TDV Dengue Cases	TDV Incidence Density	Placebo Dengue Cases	Placebo Incidence Density	Vaccine Efficacy (95% CI)
VCD cases					
Baseline Seropositive ^a	41/9,165 (0.4%)	0.5	110/4,587 (2.4%)	2.7	82.2% (74.5%-87.6%)
Baseline Seronegative ^a	20/3,531 (0.6%)	0.6	39/1,726 (2.3%)	2.5	74.9% (57.0%-85.4%)
DENV-1	16/12,700 (0.1%)	0.1	30/6,316 (0.5%)	0.5	73.7% (51.7%-85.7%)
DENV-2	3/12,700 (<0.1%)	<0.1	64/6,316 (1.0%)	1.1	97.7% (92.7%-99.3%)
DENV-3	39/12,700 (0.3%)	0.3	51/6,316 (0.8%)	0.9	62.6% (43.3%-75.4%)
DENV-4 ^d	3/12,700 (<0.1%)	<0.1	4/6,316 (<0.1%)	<0.1	63.2% (-64.6%-91.8%)
4-5 Years Old	13/1,619 (0.8%)	0.9	23/801 (2.9%)	3.2	72.8% (46.2%-86.2%)
6-11 Years Old	34/7,009 (0.5%)	0.5	85/3,491 (2.4%)	2.7	80.7% (71.3%-87.0%)
12-16 Years Old	14/4,072 (0.3%)	0.4	41/2,024 (2.0%)	2.2	83.3% (69.3%-90.9%)
Asia	54/5,894 (0.9%)	1.0	127/2,942 (4.3%)	4.9	79.5% (71.8%-85.1%)
Latin America	7/6,806 (0.1%)	0.1	22/3,374 (0.7%)	0.7	84.3% (63.1%-93.3%)
Hospitalized VCD cases					
Baseline Seropositive ^a	4/9,165 (<0.1%)	<0.1	35/4,587 (0.8%)	0.8	94.4% (84.3%-98.0%)
Baseline Seronegative ^a	1/3,531 (<0.1%)	<0.1	18/1,726 (1.0%)	1.2	97.2% (79.1%-99.6%)
Cases of DHF ^b					
All participants	1/12,700 (<0.1%)	<0.1	4/6,316 (<0.1%)	<0.1	87.3% (-13.5%-98.6%)
Severe VCD Cases ^c					
All participants	1/12,700 (<0.1%)	<0.1	1/6,316 (<0.1%)	<0.1	50.8% (-686.9%-96.9%)

VCD, virologically-confirmed dengue;

DHF, dengue hemorrhagic fever

^aSeronegative for all serotypes; baseline seropositive defined as reciprocal neutralizing antibody titer ≥ 10 to one or more serotypes.^bVCD cases meeting WHO 1997 DHF criteria; incidence density defined as the number of cases per 100 person-years at risk; percentages are based on total number (denominator) of per protocol set participants evaluated.^cTwo severe VCD were not classified as DHF.^dThe number of cases identified was sufficient to provide reasonably precise estimates of vaccine efficacy against all individual serotypes, except DENV-4.

Clinical signs and symptoms of virologically-confirmed dengue cases during Part 1 study period in safety set data are shown in Table 22.

TABLE 22

Clinical signs and symptoms of virologically-confirmed dengue cases during Part 1 study period (safety set data)			
	TDV (N = 13,380)	Placebo (N = 6,687)	Relative Risk
Number of VCD Cases	78	200	—
Median Duration of Febrile Illness (days; 95% CI) ^a	6.0 (5.7-7.4)	6.0 (5.9-6.8)	—
Median Duration of Fever (days; 95% CI)	4.0 (3.9-4.6)	5.0 (4.5-5.0)	—
Number of Hospitalized VCD Cases	9	67	—
Median Duration of Hospitalization (days; 95% CI)	5.0 (2.8-5.4)	5.0 (4.6-5.4)	—
Evidence of Bleeding (%; n/N)	3.8% (3/78)	3.5% (7/200)	1.10
Plasma Leakage (%; n/N)	2.6% (2/78)	6.5% (13/200)	0.39
Plasma Leakage - Pleural Effusion (%; n/N)	1.3% (1/78)	1.5% (3/200)	—
Plasma Leakage - Ascites (%; n/N)	1.3% (1/78)	3.0% (6/200)	—
Plasma Leakage - Radiological Signs (%; n/N)	40.0% (2/5)	19.6% (10/51)	—
Plasma Leakage - Hematocrit Increase $\geq 20\%$ (%; n/N) ^b	3.8% (2/53)	9.5% (13/137)	—

TABLE 22-continued

Clinical signs and symptoms of virologically-confirmed dengue cases during Part 1 study period (safety set data)			
	TDV (N = 13,380)	Placebo (N = 6,687)	Relative Risk
Platelet Count $\leq 100 \times 10^9$ (%), n/N ^c	6.4% (5/78)	22.0% (44/200)	0.29
Platelet Count $\leq 50 \times 10^9$ (%), n/N ^c	3.8% (3/78)	11.0% (22/200)	0.35
ALT or AST ≥ 1000 U/L (%), n/N ^c	0% (0/78)	0% (0/200)	—

VCD, virologically-confirmed dengue;

ALT, alanine aminotransferase;

AST, aspartate aminotransferase

^aDuration of febrile illness defined as start date of earliest symptom to end date of latest symptom plus one day (symptoms considered include fever and any general symptoms).^bHematocrit increase defined as maximum hematocrit between Day 3 and Day 7 inclusive, from onset of fever $\geq 20\%$ increase over minimum hematocrit before Day 3 or after Day 7 from onset of fever.^cFor platelet, ALT, and AST data, assessments within 14 days of onset of febrile illness have been considered.

N refers to number of VCD cases with available data for the specific parameter

e) Immunogenicity

The highest geometric mean titers (GMTs) were observed against DENV-2 regardless of baseline serostatus (Table 24). A very high tetravalent seropositivity rate (99.5%) in baseline seronegatives one month after the second dose (Tables 23 and 24) was observed.

Seropositivity rate (% of seropositive subjects) for each of the four dengue serotypes is determined at prevaccination on Day 1 (Month 0), post-first vaccination on Day 30 (Month 1), prevaccination on Day 90 (Month 3), post-second vaccination on Day 120 (Month 4), Day 270 (Month 9), Day

450 (Month 15), and then annually. Seropositivity rates (% participants, 95% CI) by dengue serotype per protocol set for immunogenicity data for Day 0, Day 30, Day 90, Day 120, and Day 270 are shown in Table 23.

Seropositivity rates (% participants, 95% CI) by dengue serotype against three or more serotypes (trivalent) and against all four serotypes (tetravalent) per protocol set for immunogenicity data for Day 0, Day 30, Day 90, Day 120, and Day 270 are shown in Table 23. The tetravalent seropositivity rates were high (>91%) in baseline seronegatives six months after second dose.

TABLE 23

Seropositivity rates (% participants, 95% CI) by dengue serotype (per protocol set for immunogenicity data)			
BASELINE SEROPOSITIVE		BASELINE SERONEGATIVE	
TDV N = 1,816	Placebo N = 902	TDV N = 702	Placebo N = 345
DENV-1			
89.1 (87.6-90.5)	90.6 (88.5-92.4)	0 (0-0.5)	0 (0-1.1)
99.5 (99.1-99.8)	88.6 (86.3-90.7)	94.1 (92.0-95.8)	4.9 (2.8-7.8)
99.3 (98.8-99.6)	90.2 (88.1-92.1)	91.6 (89.3-93.5)	6.1 (3.8-9.2)
>99.9 (99.7-100)	90.3 (88.1-92.3)	99.5 (98.6-99.9)	8.3 (5.5-11.9)
99.6 (99.1-99.8)	89.8 (87.5-91.8)	95.1 (93.0-96.6)	9.0 (6.0-12.8)
DENV-2			
96.5 (95.6-97.3)	97.2 (95.9-98.2)	0 (0-0.5)	0 (0-1.1)
99.9 (99.6-100)	93.3 (91.4-94.9)	98.6 (97.4-99.4)	10.7 (7.5-14.5)
>99.9 (99.7-100)	94.0 (92.2-95.5)	99.0 (98.0-99.6)	12.2 (8.9-16.1)
99.9 (99.6-100)	93.6 (91.7-95.2)	100 (99.4-100)	14.7 (11.0-19.1)
100 (99.8-100)	94.6 (92.8-96.1)	100 (99.4-100)	18.3 (14.1-23.2)
DENV-3			
88.1 (86.5-89.6)	88.0 (85.7-90.1)	0 (0-0.5)	0 (0-1.1)
99.8 (99.4-99.9)	87.6 (85.1-89.7)	96.1 (94.3-97.4)	4.0 (2.1-6.7)
99.5 (99.1-99.8)	87.3 (84.9-89.4)	94.4 (92.5-96.0)	2.0 (0.8-4.1)
99.8 (99.5-100)	87.9 (85.5-90.1)	100 (99.4-100)	5.1 (2.9-8.2)
99.7 (99.4-99.9)	87.1 (84.6-89.4)	96.4 (94.6-97.7)	7.7 (4.9-11.3)
DENV-4			
88.1 (86.5-89.6)	87.4 (85.0-89.5)	0 (0-0.5)	0 (0-1.1)
99.6 (99.2-99.9)	86.6 (84.1-88.8)	90.5 (88.0-92.6)	1.8 (0.7-3.9)
99.3 (98.8-99.7)	86.9 (84.5-89.0)	92.0 (89.8-93.9)	2.9 (1.4-5.3)
>99.9 (99.7-100)	88.3 (85.9-90.4)	99.8 (99.1-100)	4.8 (2.7-7.8)
99.7 (99.3-99.9)	87.6 (85.1-89.9)	97.0 (95.4-98.2)	6.3 (3.9-9.7)
Three or More Serotypes			
87.5 (85.9-89.0)	87.3 (84.9-89.4)	0 (0-0.5)	0 (0-1.1)
99.8 (99.5-100)	87.2 (84.7-89.4)	96.5 (94.8-97.8)	1.2 (0.3-3.1)
99.7 (99.3-99.9)	87.7 (85.3-89.7)	94.9 (93.0-96.4)	1.7 (0.6-3.7)

TABLE 23-continued

Seropositivity rates (% participants, 95% CI) by dengue serotype (per protocol set for immunogenicity data)			
BASELINE SEROPOSITIVE		BASELINE SERONEGATIVE	
TDV N = 1,816	Placebo N = 902	TDV N = 702	Placebo N = 345
99.9 (99.6-100)	88.4 (86.0-90.5)	99.8 (99.1-100)	4.2 (2.2-7.0)
99.7 (99.4-99.9)	87.3 (84.7-89.5)	97.5 (96.0-98.6)	5.7 (3.3-8.9)
All Four Serotypes			
83.5 (81.7-85.2)	83.5 (80.9-85.8)	0 (0-0.5)	0 (0-1.1)
99.1 (98.5-99.5)	82.9 (80.2-85.4)	85.3 (82.4-87.9)	0.9 (0.2-2.6)
98.6 (97.9-99.1)	83.6 (81.0-86.0)	84.3 (81.4-86.9)	1.4 (0.5-3.3)
99.8 (99.5-100)	85.2 (82.6-87.6)	99.5 (98.6-99.9)	3.5 (1.8-6.2)
99.2 (98.7-99.6)	84.6 (81.9-87.0)	91.3 (88.7-93.4)	5.3 (3.1-8.5)

Seropositivity rates (% participants, 95% CI) by dengue serotype (per protocol set for immunogenicity data; seropositive defined as a reciprocal neutralizing antibody titer ≥ 10 ; baseline seronegative defined as seronegative to all serotype; baseline seropositive defined as seropositive to one or more serotypes; N refers to number of participants in the analysis set; number of participants evaluated at each timepoint may vary)

Geometric mean titers (GMTs) of neutralizing antibodies (microneutralization test [MNT]) for each dengue serotype are determined at pre-vaccination on Day 1 (Month 0), post-first vaccination on Day 30 (Month 1), pre-vaccination on Day 90 (Month 3), post-second vaccination on Day 120 (Month 4), Day 270 (Month 9), Day 450 (Month 15), and then annually. Geometric mean titers (95% CI) by dengue serotype per protocol set for immunogenicity data for Day 0, Day 30, Day 90, Day 120, and Day 270 are shown in Table 24.

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Vaccine viremia is assessed by three PCRs: dengue detection RT-PCR, vaccine screening PCR and TDV sequencing in subjects with febrile illness within 30 days after each vaccination.

f) Safety

Rates of serious adverse events (SAEs) were similar in the vaccine and placebo groups (3.1% and 3.8% of participants, respectively; Table 25). One vaccine and four placebo recipients experienced SAEs considered to be related to receiving blinded investigational product by the investigator (two experienced hypersensitivity, two were diagnosed with

TABLE 24

Geometric mean titers (95% CI) by dengue serotype (per protocol set for immunogenicity data)				
BASELINE SEROPOSITIVE		BASELINE SERONEGATIVE		
TDV N = 1,816	Placebo N=902	TDV N = 702	Placebo N = 345	
DENV-1				
Day 1 410 (365-461)	445 (377-524)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	
Day 30 2,404 (2,204-2,622)	430 (361-512)	118 (106-131)	5.8 (5.3-6.3)	
Day 90 1,945 (1,791-2,112)	410 (349-481)	91 (82-102)	5.9 (5.4-6.3)	
Day 120 2,115 (1,957-2,286)	451 (381-534)	184 (169-201)	6.3 (5.7-7.0)	
Day 270 1,447 (1,329-1,574)	415 (350-492)	87 (79-97)	6.3 (5.7-6.9)	
DENV-2				
Day 1 745 (674-825)	802 (697-924)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	
Day 30 6,697 (6,301-7,117)	744 (635-870)	6,277 (5,648-6,977)	6.6 (6.0-7.3)	
Day 90 4,826 (4,571-5,096)	729 (629-845)	1,682 (1,544-1,834)	7.0 (6.3-7.9)	
Day 120 4,897 (4,646-5,163)	766 (655-896)	1,730 (1,614-1,855)	7.7 (6.7-8.8)	
Day 270 3,692 (3,496-3,898)	776 (665-906)	929 (856-1,010)	8.7 (7.4-10.2)	
DENV-3				
Day 1 357 (321-398)	356 (305-415)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	
Day 30 2,255 (2,094-2,428)	349 (298-409)	194 (173-218)	5.5 (5.2-5.9)	
Day 90 1,563 (1,453-1,682)	321 (277-374)	94 (85-104)	5.5 (5.1-5.9)	
Day 120 1,761 (1,646-1,885)	353 (301-414)	228 (212-246)	6.0 (5.4-6.6)	
Day 270 1,089 (1,009-1,175)	307 (261-360)	72 (66-78)	6.3 (5.7-7.0)	
DENV-4				
Day 1 218 (198-241)	234 (203-270)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	
Day 30 1,303 (1,221-1,391)	222 (191-258)	111 (98-125)	5.4 (5.0-5.7)	
Day 90 1,002 (940-1,069)	215 (187-248)	63 (57-70)	5.5 (5.1-5.9)	
Day 120 1,129 (1,066-1,196)	241 (208-280)	144 (134-155)	5.8 (5.3-6.4)	
Day 270 778 (730-830)	229 (197-266)	64 (59-70)	6.2 (5.6-6.9)	

dengue, and one with DHF). There were five deaths during Part 1, and all were considered unrelated to the investigational product or study procedures. Total rates of unsolicited AEs were similar between the vaccine and placebo groups. The most commonly ($\geq 1\%$ of vaccine-recipients) reported unsolicited AEs within four weeks of any dose by preferred term were pyrexia (vaccine group 1.5%; placebo 1.4%), nasopharyngitis (vaccine 2.7%; placebo 3.0%), upper respiratory tract infection (vaccine 2.6%; placebo 2.9%), and viral infection (vaccine 1.1%; placebo 0.9%). Solicited local reactions were reported more frequently in the vaccine group.

3. The unit dose of item 1, wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent:
 - (i) has a concentration of $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.0 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (ii) has a concentration of $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $4.9 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (iii) has a concentration of $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.7 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
 - (iv) has a concentration of $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.5 \log 10 \text{ pfu}/0.5 \text{ mL}$.

TABLE 25

Overview of safety data. Subjects with at least one adverse event after any vaccine dose. Data presented as number of events (percentage of subjects; number [n] of subjects/total [N] subjects) unless otherwise stated (safety set data)

	TDV	Placebo
Safety Set	N = 13,380	N = 6,687
SAEs	3.1% (409/13,380)	3.8% (255/6,687)
Non-IP-Related ^a SAEs	3.0% (408/13,380)	3.8% (251/6,687)
IP-Related ^a SAEs	<0.1% (1/13,380)	<0.1% (4/6,687)
SAEs Leading to IP Withdrawal and/or Trial Discontinuation	0.1% (18/13,380)	0.1% (8/6,687)
Deaths	<0.1% (4/13,380)	<0.1% (1/6,687)
IP-Related Deaths	0% (0/13,380)	0% (0/6,687)
Safety Subset	N = 2,663	N = 1,329
Unsolicited AEs Occurring Within 4 Weeks of Any Dose	18.4% (490/2,663)	18.8% (250/1,329)
IP-Related ^a Unsolicited AEs Occurring Within 4 Weeks of Any Dose	1.0% (27/2,663)	1.6% (21/1,329)
Solicited Systemic AEs Occurring Within 2 Weeks of Any Dose ^b	42.0% (1,107/2,635)	38.0% (501/1,317)
IP-Related ^a Solicited Systemic AEs Occurring Within 2 Weeks of Any Dose	31.2% (821/2,635)	28.2% (371/1,317)
Solicited Local Reactions Occurring Within 1 Week of Any Dose ^c	36.7% (967/2,633)	25.7% (338/1,317)

AE, adverse event;

SAE, serious adverse event;

IP, investigational product/TDV

^aIP-related, defined as related to the investigational product as assessed by investigator

^bonly participants with diary data available were evaluated

^call injection site (solicited local) reactions considered to be IP-related

LIST OF ITEMS OF THE INVENTION

1. A unit dose of a dengue vaccine composition comprising: a tetravalent dengue virus composition including four live, attenuated dengue virus strains wherein the unit dose is lyophilized and upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent comprises:
 - (i) a chimeric dengue serotype 2/1 strain in a concentration of at least $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (ii) a dengue serotype 2 strain in a concentration of at least $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (iii) a chimeric dengue serotype 2/3 strain in a concentration of at least $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
 - (iv) a chimeric dengue serotype 2/4 strain in a concentration of at least $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$.
2. The unit dose of item 1, wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent
 - (i) has a concentration of $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.0 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (ii) has a concentration of $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $4.9 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (iii) has a concentration of $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.7 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
 - (iv) has a concentration of $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $6.2 \log 10 \text{ pfu}/0.5 \text{ mL}$.
3. The unit dose of item 1, wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent
 - (i) has a concentration of $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.0 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (ii) has a concentration of $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $4.9 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (iii) has a concentration of $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.7 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
 - (iv) has a concentration of $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.5 \log 10 \text{ pfu}/0.5 \text{ mL}$.
4. The unit dose of any one of items 1 to 3, wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent
 - (i) has a concentration of $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $3.6 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (ii) has a concentration of $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$,
 - (iii) has a concentration of $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $4.6 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
 - (iv) has a concentration of $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$ to $5.1 \log 10 \text{ pfu}/0.5 \text{ mL}$.
5. The unit dose of any one of items 1 to 4, wherein upon reconstitution with a pharmaceutically acceptable diluent
 - (i), (ii), (iii), and (iv) provide a total concentration of $\text{pfu}/0.5 \text{ mL}$ and based on said total concentration the concentration of (ii) in $\text{pfu}/0.5 \text{ mL}$ is less than 10% or less than 8%, and the concentration of (iv) in $\text{pfu}/0.5 \text{ mL}$ is at least 50%.
6. The unit dose of item 5, wherein upon reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of $\text{pfu}/0.5 \text{ mL}$ and based on said total concentration the concentration of (i) in $\text{pfu}/0.5 \text{ mL}$ is at least 1%, and the concentration of (iii) in $\text{pfu}/0.5 \text{ mL}$ is at least 7% or at least 8%.
7. The unit dose of any one of items 1 to 6, wherein upon reconstitution with a pharmaceutically acceptable diluent

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(i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 mL and based on said total concentration the concentration of (i) in pfu/0.5 mL is 1% to 7% of the total concentration, (ii) in pfu/0.5 mL is less than 8% of the total concentration, such as in the range of 1% to 8% of the total concentration, (iii) in pfu/0.5 mL is at least 10% of the total concentration, and (iv) in pfu/0.5 mL is at least 65% of the total concentration, such as in the range of 65% to 80%.

8. The unit dose of any one of items 1 to 7, wherein the arithmetic sum of all four serotypes is in the range of 4.6 log 10 pfu/0.5 mL to 6.7 log 10 pfu/0.5 mL, preferably in the range of 4.6 log 10 pfu/0.5 mL to 5.5 log 10 pfu/0.5 mL.

9. The unit dose of any one of items 1 to 8, wherein reconstitution with a pharmaceutically acceptable diluent is made with 0.5 ml of the pharmaceutically acceptable diluent.

10. The unit dose of any one of items 1 to 9, wherein the lyophilized unit dose is prepared from a solution further comprising a non-reducing sugar, a surfactant, a protein and an inorganic salt.

11. The unit dose of item 10, wherein the non-reducing sugar is trehalose, the surfactant is poloxamer 407, the protein is human serum albumin and the inorganic salt is sodium chloride.

12. The unit dose of item 10 or 11, wherein the solution comprises:
from about 10% (w/v) to about 20% (w/v) α,α -trehalose dihydrate or an equimolar amount of other forms of α,α -trehalose,
from about 0.5% (w/v) to about 1.5% (w/v) poloxamer 407,
from about 0.05% (w/v) to about 2% (w/v) human serum albumin, and
from about 70 mM to about 140 mM sodium chloride.

13. The unit dose of any one of items 1 to 12, wherein the lyophilized unit dose is prepared from a solution comprising:
about 15% (w/v) α,α -trehalose dihydrate,
about 1% (w/v) poloxamer 407,
about 0.1% (w/v) human serum albumin, and
and about 100 mM sodium chloride.

14. The unit dose of any one of items 1 to 13, wherein each one of the four live attenuated dengue virus strains has attenuating mutations in the 5'-noncoding region (NCR) at nucleotide 57 from cytosine to thymidine, in the NS1 gene at nucleotide 2579 from guanine to adenine resulting in an amino acid change at position 828 from glycine to asparagine, and in the NS3 gene at nucleotide 5270 from adenine to thymine resulting in an amino acid change at position 1725 from glutamine to valine, preferably further comprising one or more of the mutations selected from the list comprising:

a) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymidine resulting in an amino acid at position 1308 from leucine to phenylalanine,

b) a silent mutation in the NS3 gene at nucleotide 5547 from thymidine to cytosine, and

c) a mutation in the NS4A gene at nucleotide 6599 from guanine to cytosine resulting in an amino acid change at position 2168 from glycine to alanine.

15. The unit dose of item 14, wherein (i) further comprises one or more of the mutations selected from the list comprising:

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a mutation in the NS2A gene at nucleotide 3823 from adenine to cytosine resulting in an amino acid change at position 1243 from isoleucine to leucine,
a mutation in the NS2B gene at nucleotide 4407 from adenine to thymidine resulting in an amino acid change at position 1437 from glutamine to asparagine, and
a silent mutation in the NS4B gene at nucleotide 7311 from adenine to guanine.

16. The unit dose of item 14 or 15, wherein (ii) further comprises one or more of the mutations selected from the list comprising:

a mutation in the prM gene at nucleotide 592 from adenine to guanine resulting in an amino acid change at position 166 from lysine to glutamine, and

a mutation in the NS5 gene at nucleotide 8803 from adenine to guanine resulting in an amino acid change at position 2903 from isoleucine to valine.

17. The unit dose of any one of items 14 to 16, wherein (iii) further comprises one or more of the mutations selected from the list comprising:

a mutation in the E gene at nucleotide 1603 from adenine to thymidine resulting in an amino acid change at position 503 from threonine to serine, and

a silent mutation in the NS5 gene at nucleotide 7620 from adenine to guanine.

18. The unit dose of any one of items 14 to 17, wherein (iv) further comprises one or more of the mutations selected from the list comprising

a silent mutation in the C gene at nucleotide 225 from adenine to thymidine,

a mutation in the NS2A gene at nucleotide 3674 from adenine to guanine resulting in an amino acid change at position 1193 from asparagine to glycine,

a mutation in the NS2A gene at nucleotide 3773 from adenine to an adenine/guanine mix resulting in an amino acid change at position 1226 from lysine to a lysine/asparagine mix,

a silent mutation in the NS3 gene at nucleotide 5391 from cytosine to thymidine,

a mutation in the NS4A gene at nucleotide 6437 from cytosine to thymidine resulting in an amino acid change at position 2114 from alanine to valine,

a silent mutation in the NS4B gene at nucleotide 7026 from thymidine to a thymidine/cytosine mix, and

a silent mutation in the NS5 gene at nucleotide 9750 from adenine to cytosine.

19. The unit dose of any one of items 1 to 18, wherein

(i) has the amino acid sequence of SEQ ID NO. 2,
(ii) has the amino acid sequence of SEQ ID NO. 4,
(iii) has the amino acid sequence of SEQ ID NO. 6, and
(iv) has the amino acid sequence of SEQ ID NO. 8.

20. The unit dose of any one of items 1 to 19 reconstituted with 0.3 to 0.8 mL of liquid for reconstitution.

21. The unit dose of item 20 reconstituted with 0.5 mL of liquid for reconstitution.

22. The unit dose of item 20 or 21, wherein the liquid for reconstitution is 37 mM aqueous sodium chloride solution.

23. A kit for preparing a reconstituted unit dose comprising the following components:

a) a unit dose of any one of items 1 to 19, and
b) a pharmaceutically acceptable diluent for reconstitution.

24. The kit of item 23, wherein the pharmaceutically acceptable diluent for reconstitution is 37 mM sodium chloride.

25. Container, such as a vial, comprising one to ten unit doses of any one of items 1 to 22.

26. A method of preventing dengue disease in a subject population comprising administering to the subject population a reconstituted unit dose of any one of items 20 to 22.
27. A method of preventing virologically confirmable dengue disease in a subject population comprising administering to the subject population a reconstituted unit dose of a tetravalent dengue virus composition including four live, attenuated dengue virus strains.
28. A method of preventing virologically confirmable dengue disease with hospitalization in a subject population comprising administering to the subject population a reconstituted unit dose of a tetravalent dengue virus composition including four live, attenuated dengue virus strains.
29. The method of items 26 to 28, wherein the geometric mean neutralizing antibody titers (GMTs) of the subject population when tested in at least 40, or at least 50, or at least 60 subjects at day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, provide a ratio of not more than 50, or not more than 40, or nor more than 30, or not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 4.
30. The method of item 29, wherein said GMTs of the subject population further provide a ratio of not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 1, and/or a ratio of not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 3.
31. A method of preventing dengue disease in a subject comprising administering to the subject a reconstituted unit dose of any one of items 20 to 22.
32. A method of preventing virologically confirmable dengue disease in a subject comprising administering to the subject a reconstituted unit dose of a tetravalent dengue virus composition including four live, attenuated dengue virus strains.
33. A method of preventing virologically confirmable dengue disease with hospitalization in a subject comprising administering to the subject a reconstituted unit dose of a tetravalent dengue virus composition including four live, attenuated dengue virus strains.
34. The method of items 31 to 33, wherein the neutralizing antibody titers of the subject when tested at day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, provide a ratio of not more than 50, or not more than 40, or nor more than 30, or not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4.
35. The method of item 34, wherein said neutralizing antibody titers of the subject further provide a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 1, and/or a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 3.
36. The method of any one of items 26 to 35, wherein the method is for preventing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).
37. The method of any one of items 26 to 36, wherein the reconstituted unit dose is administered by subcutaneous injection, preferably to the deltoid region of the arm.

38. The method of any one of items 26 to 37, wherein two reconstituted unit doses of any one of items 20 to 22 are administered within 12 months or more.
39. The method of any one of items 26 to 37, wherein two reconstituted unit doses of any one of items 20 to 22 are administered within six months, preferably within three months.
40. The method of item 39, wherein the two reconstituted unit doses are administered at day 0 and day 90 or at day 1 and day 90.
41. The method of item 38 to 40, wherein a third unit dose is administered after administration of the second unit dose, preferably within 12 months after administration of the first unit dose.
42. The method of item 38 to 40, wherein a third unit dose is administered after administration of the second unit dose, preferably within 12 months after administration of the second unit dose.
43. The method of any one of items 26 to 42, wherein the subject or subject population is seronegative with respect to all dengue serotypes.
44. The method of any one of items 26 to 42, wherein the subject population or subject is seropositive with respect to at least one dengue serotype.
45. The method of any one of items 26 to 44, wherein the subject or subject population is of 2 to 60 years of age.
46. The method of any one of items 26 to 44, wherein the subject or subject population is of 2 to 17 years of age.
47. The method of any one of items 26 to 44, wherein the subject or subject population is under 9 years of age, under 4 years of age, or under 2 years of age or from 2 to 9 years of age, or from 2 to 5 years of age, or from 4 to 9 years of age or from 6 to 9 years of age, and optionally wherein the subject is seronegative with respect to all dengue serotypes.
48. The method of any one of items 26 to 44, wherein the subject or subject population is of 4 to 16 years of age.
49. The method of item 48, wherein the subject or subject population is of 4 to 5 years of age.
50. The method of item 48, wherein the subject or subject population is of 6 to 11 years of age.
51. The method of item 48, wherein the subject or subject population is of 12 to 16 years of age.
52. The method of any one of items 26 to 51, wherein the subject or subject population is from a dengue endemic region.
53. The method of any one of items 26 to 51, wherein the subject or subject population is from a dengue non-endemic region.
54. The method of any one of items 52 or 53, wherein the subject or subject population is from Asia Pacific or Latin America.
55. The method of any one of items 26 to 54, wherein the subject or subject population has been subject to prior vaccination against Yellow Fever, wherein prior vaccination against Yellow Fever refers to a vaccination prior to the second administration or prior to the first administration.
56. The method of any one of items 26 to 54, wherein the subject or subject population is has been subject to prior vaccination against Japanese Encephalitis, wherein prior vaccination against Japanese Encephalitis refers to a vaccination prior to the second administration or prior to the first administration.
57. The method of any one of items 26 to 54, wherein the subject or subject population is has not been subject to prior vaccination against Yellow Fever.

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58. The method of any one of items 26 to 54, wherein the subject or subject population is has not been subject to prior vaccination against Japanese Encephalitis.
59. The method of any one of items 26 to 58 having a combined vaccine efficacy against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule.
60. The method of item 59, wherein the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 55%, is more than 60%, is more than 65%, is more than 70% or is more than 72%.
61. The method of any one of items 26 to 60 having a combined vaccine efficacy against all four serotypes of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.
62. The method of item 61, wherein the combined vaccine efficacy against all four serotypes is more than 40%, is more than 50%, is more than 55%, is more than 60%, is more than 65%, is more than 70%, is more than 75% is more than 78%, is more than 79% or is about 80%.
63. The method of any one of items 26 to 62 having a combined relative risk against all four serotypes with a 2-sided 95% confidence interval, wherein the upper bound is less than 0.75, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.
64. The method of item 63, wherein the upper bound is less than 0.70, is less than 0.65, is less than 0.60, is less than 0.55, is less than 0.50, is less than 0.45, is less than 0.40, is less than 0.35, is less than 0.30 or is less than 0.28.
65. The method of any one of items 26 to 64, wherein the combined relative risk against all four serotypes is less than 0.70, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.
66. The method of item 65, wherein the combined relative risk against all four serotypes is less than 0.65, is less than 0.60, is less than 0.55, is less than 0.50, is less than 0.45, is less than 0.40, is less than 0.35, is less than 0.30, is less than 0.25 or is less than 0.23.
67. The method of any one of items 26 to 58 having a combined vaccine efficacy against all four serotypes with a 2-sided 95% confidence interval, wherein the lower

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- bound is more than 61.0%, or more than 65.0% or more than 70.0% or more than 72.0% when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects from endemic irrespective of serostatus at baseline and being selected from the group consisting of 4 to 16 year old subjects at the time of randomization, wherein said unit dose or said placebo is administered at least twice within 6 months or less, about 30 days after the last administration of the administration schedule until at least 12 or 13 months after the last administration of the administration schedule.
68. The method of any one of items 26 to 58 having a combined vaccine efficacy against all four serotypes of more than 66%, or of more than 70%, or of more than 75%, or of more than 77%, or of more than 80%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects from endemic areas irrespective of serostatus at baseline and being selected from the group consisting of 4 to 16 year old subjects at the time of randomization, wherein said unit dose or said placebo is administered at least twice within 6 months or less, about 30 days after the last administration of the administration schedule until at least 12 months or 13 month after the last administration of the administration schedule.
69. The method of item 67 or 68, wherein the combined vaccine efficacy against all four serotypes is measured about 30 days after the last administration of the administration schedule until 12 or 13 months after the last administration of the administration schedule.
70. The method of item 67 or 68, wherein said unit dose or said placebo is administered twice within three months, in particular at about day 1 and about day 90, and wherein the combined vaccine efficacy against all four serotypes is measured 30 days after the second administration until 12 or 13 months after the second administration of the administration schedule.
71. The method of any one of items 26 to 70 being effective and safe.
72. The method of any one of items 26 to 71 having a relative risk for virologically confirmed dengue with hospitalization which is 1 or less, or 0.8 or less, or 0.6 or less, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects.
73. The method of any one of items 59 to 72, wherein the healthy subjects of the subject population are of 4 to 16 years of age at the time of randomization.
74. The method of any one of items 59 to 72, wherein the healthy subjects of the subject population are of 4 to 5 years of age at the time of randomization.
75. The method of any one of items 59 to 72, wherein the healthy subjects of the subject population are of 6 to 11 years of age at the time of randomization.
76. The method of any one of items 59 to 72, wherein the healthy subjects of the subject population are of 12 to 16 years of age at the time of randomization.
77. The method of any one of items 59 to 72, wherein the healthy subjects of the subject population are from Asia Pacific or Latin America.
78. The method of any one of items 59 to 77, wherein the healthy subjects of the subject population are seropositive with respect to at least one serotype at baseline.

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79. The method of any one of items 59 to 77, wherein the healthy subjects of the subject population are seronegative with respect to all serotypes at baseline.
80. The method of any one of items 59 to 79, wherein the healthy subjects of the subject population have been subject to prior vaccination against Yellow Fever.
81. The method of any one of items 59 to 79, wherein the healthy subjects of the subject population have been subject to prior vaccination against Japanese Encephalitis.
82. The method of any one of items 59 to 79, wherein the healthy subjects of the subject population have not been subject to prior vaccination against Yellow Fever.
83. The method of any one of items 59 to 79, wherein the healthy subjects of the subject population have not been subject to prior vaccination against Japanese Encephalitis.
84. A method for stimulating an immune response to all four serotypes of dengue virus in a subject, comprising administering to the subject a reconstituted unit dose of items 20 to 22.
85. The method of item 84, wherein the immune response to all four serotypes of dengue virus is balanced.
86. The method of item 84 or 85, wherein the reconstituted unit dose is administered by subcutaneous injection, preferably to the deltoid region of the arm.
87. The method of any one of items 84 to 86, wherein two unit doses of any one of items 20 to 22 are administered within 12 months or more.
88. The method of any one of items 84 to 87, wherein two reconstituted unit doses of any one of items 20 to 22 are administered within six months, preferably within three months.
89. The method of item 88, wherein the two reconstituted unit doses are administered at day 0 and day 90 or at day 1 and day 90.
90. The method of item 87 to 89, wherein a third unit dose is administered after the administration of the second unit dose, preferably within 12 month of administration of the first unit dose.
91. The method of item 87 to 89, wherein a third unit dose is administered after the administration of the second unit dose, preferably within 12 month of administration of the second unit dose.
92. The method of any one of items 84 to 91, wherein the subject is from a dengue endemic region.
93. The method of any one of items 84 to 91, wherein the subject is from a dengue non-endemic region.
94. The method of any one of items 84 to 93, wherein the subject is seronegative with respect to all dengue serotypes.
95. The method of any one of items 84 to 93, wherein the subject is seropositive with respect to at least one dengue serotype.
96. The method of any one of items 84 to 95, wherein the neutralizing antibody titers of the subject when tested at day 180 or day 365 after at least a first administration of said reconstituted unit dose, and optionally a second administration of said reconstituted unit dose 90 days after said first administration, provide a ratio of not more than 50, or not more than 40, or nor more than 30, or not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4.
97. The method of item 96, wherein said neutralizing antibody titers of the subject further provide a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 1, and/or a ratio of not more than 20 for the

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- neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 3.
98. The method of any one of items 84 to 97, wherein the subject is of 2 and 60 years of age.
99. The method of any one of items 84 to 97, wherein the subject is under 9 years of age, under 4 years of age, or under 2 years of age.
100. The method of any one of items 26 to 99, wherein the reconstituted unit dose is obtained from the kit according to item 23 or 24.
101. The reconstituted unit dose of any one of item 20 to 22 for use in a method of items 26 to 100.
102. Use of a reconstituted unit dose of any one of items 20 to 22 for the manufacture of a medicament for a method according to items 26 to 100.
103. A method for determining the titer of neutralizing antibodies against each of dengue serotypes 1, 2, 3 and 4 in a blood serum sample, the method comprising the steps of:
- (a) seeding cells from a dengue-susceptible cell line on 96-well assay plates and culturing the cells for a culture period;
 - (b) preparing serial dilutions of the blood serum sample;
 - (c) separately mixing the serially diluted blood serum samples prepared in step (b) with dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4 to obtain separate mixtures for each dengue serotype and incubating the separate mixtures;
 - (d) adding the separate mixtures prepared in (c) to the cells seeded and cultured in step (a) and incubating the cells with the separate mixtures;
 - (e) providing an overlay for the inoculated cells and incubating the cells for an incubation period of 40 to 75 hours;
 - (f) determining the number of plaques in each well and comparing the number of plaques in each well to a control to determine the level of neutralizing antibodies against each of dengue serotypes 1, 2, 3 and 4.
104. Method according to item 103, wherein in step (e) different incubation periods are used for the mixtures of different dengue serotypes.
105. Method according to item 103 or 104 wherein in step (e) the incubation period for mixtures of dengue serotype 4 is shorter than the incubation period for mixtures of dengue serotypes 1, 2 and 3.
106. Method according to item 105, wherein the incubation period for mixtures of dengue serotype 4 is 46 ± 2 hours.
107. Method according to any one of the items 103 to 106, wherein in step (e) the incubation period for mixtures of dengue serotype 2 is longer than the incubation period for mixtures of dengue serotypes 1, 3 and 4.
108. Method according to item 107, wherein the incubation period for mixtures of dengue serotype 2 is 70 ± 2 hours.
109. Method according to any one of items 103 to 108, wherein the dengue-susceptible cell line is selected from Vero cells, LLC-MK2 cells and BHK-21 cells.
110. Method according to any one of items 103 to 109, wherein the culture period in step (a) is 12 to 36 hours.
111. Method according to any one of items 103 to 110 wherein in step (c) the dengue serotype 1 is DENV-1 strain 16007, dengue serotype 2 is DENV-2 strain 16681, dengue serotype 3 is DENV-3 strain 16562 and dengue serotype 4 is DENV-4 strain 1036.
112. Method according to any one of items 103 to 111, wherein the separate mixtures in step (c) are incubated overnight at a temperature of $2^\circ C$. to $8^\circ C$.

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113. Method according to any one of items 103 to 112, wherein the overlay in step (e) is selected from the group consisting of methylcellulose, carboxymethylcellulose and agarose.
114. Method according to any one of items 103 to 113, wherein in step (e) the cells are incubated at a temperature of 33° C. to 35° C.
115. Method according to any one of items 103 to 114, wherein the number of plaques in each well is determined using serotype-specific anti-dengue monoclonal antibodies.
116. A method for determining the titer of neutralizing antibodies against each of dengue serotypes 1, 2, 3 and 4 in a blood serum sample, the method comprising the steps of:
- (a) seeding Vero cells on 96-well assay plates and culturing the Vero cells for a period of 20 to 30 hours;
 - (b) preparing serial dilutions of the serum sample;
 - (c) separately mixing the serially diluted serum samples with dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4 to prepare separate mixtures and incubating the separate mixtures overnight at a temperature of 2° C. to 8° C.;
 - (d) incubating the cells seeded and cultured in step (a) with the separate mixtures prepared in step (c) in separate wells for 90 to 120 minutes;
 - (e) providing a methylcellulose overlay for the inoculated cells and incubating the cells for an incubation period of 40 to 75 hours at 34° C.;
 - (f) determining the number of plaques in each well using serotype-specific anti-dengue monoclonal antibodies and comparing the number of plaques in each well to a control to determine the level of neutralizing antibodies against each of dengue serotypes 1, 2, 3 and 4.
117. Use of the method according to any one of items 103 to 116 for determining the dengue serostatus of a subject before vaccination with a dengue virus vaccine or for analyzing a subjects immune response after vaccination with a dengue virus vaccine.

LIST OF FURTHER ITEMS OF THE
INVENTION

1. A dengue vaccine composition for use in a method of preventing virologically confirmable dengue disease in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, and wherein the dengue vaccine composition is a tetravalent dengue virus composition including four live, attenuated dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, wherein the attenuated dengue virus strains comprise chimeric dengue viruses and at least one non-chimeric dengue virus, and wherein the dengue serotype 1 and the dengue serotype 2 are present each in a concentration based on the total concentration in pfu/0.5 mL which is within 5%-points of each other and/or are together less than about 10% of the total concentration in pfu/0.5 mL.
2. The composition for use of item 1, wherein the method does not comprise a determination of a previous dengue infection in the subject before the administration of the first unit dose of the tetravalent dengue virus composition and wherein the method is safe and effective.

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3. The composition for use of item 1 or 2, wherein the dengue serotype 3 is at least about 10% of the total concentration in pfu/0.5 mL and/or wherein the dengue serotype 4 is at least about 70% of the total concentration in pfu/0.5 mL.
4. The composition for use of any one of items 1 to 3, wherein the dengue serotype 4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 and dengue serotype 2 each represent lower concentrations than the concentration of serotype 3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.
5. The composition for use of any one of items 1 to 4, wherein the dengue serotype 1 is a chimeric dengue serotype 2/1 strain, the dengue serotype 2 is a non-chimeric dengue serotype 2 strain, the dengue serotype 3 is a chimeric dengue serotype 2/3 strain and the dengue serotype 4 is a chimeric dengue serotype 2/4 strain.
6. The composition for use of any one of items 1 to 5, wherein the dengue serotype 1 has the amino acid sequence of SEQ ID NO. 2, the dengue serotype 2 has the amino acid sequence of SEQ ID NO. 4, the dengue serotype 3 has the amino acid sequence of SEQ ID NO. 6, and the dengue serotype 4 has the amino acid sequence of SEQ ID NO. 8.
7. The composition for use of any one of items 1 to 6, wherein the unit dose upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent
 - (i) dengue serotype 1 has a concentration of 3.3 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,
 - (ii) dengue serotype 2 has a concentration of 2.7 log 10 pfu/0.5 mL to 4.9 log 10 pfu/0.5 mL,
 - (iii) dengue serotype 3 has a concentration of 4.0 log 10 pfu/0.5 mL to 5.7 log 10 pfu/0.5 mL, and
 - (iv) dengue serotype 4 has a concentration of 4.5 log 10 pfu/0.5 mL to 6.2 log 10 pfu/0.5 mL.
8. The composition for use of any one of items 1 to 7, wherein the composition further comprises about 15 (w/v) α,α-trehalose dihydrate, about 1% (w/v) poloxamer 407, about 0.1% (w/v) human serum albumin, and about 100 mM sodium chloride when measured in 0.5 ml.
9. The composition for use of any one of items 1 to 8, wherein the unit doses are administered to the deltoid region of the arm.
10. The composition for use of any one of items 1 to 9, wherein the subject is seronegative to all dengue serotypes at baseline and/or is under 9 years of age.
11. The composition for use of any one of items 1 to 10, wherein the subject is 4 to 5 years of age or 6 to 11 years of age or 12 to 16 years of age.
12. The composition for use of any one of items 1 to 11, wherein the method is for preventing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).
13. The composition for use of any one of items 1 to 12, wherein the subject is from a dengue endemic region.
14. The composition for use of any one of items 1 to 12, wherein the subject is from a dengue non-endemic region.
15. The composition for use of any one of items 1 or 14, wherein the subject is from Asia Pacific or Latin America.
16. The composition for use of any one of items 1 to 15, wherein the composition provides a seropositivity rate when it is administered to a subject population of at least

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- 50 subjects in two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population are seronegative to all dengue serotypes at baseline.
17. The composition for use of item 16, wherein at least one month after administration of the first unit dose, such as at day 30, at least 80% of the subject population are seropositive for all four dengue serotypes.
18. The composition for use of item 16 or 17, wherein before or at the time of the administration of the second unit dose, such as at day 90, at least 80% of the subject population are seropositive for all four dengue serotypes.
19. The composition for use of any one of items 16 to 18, wherein after the administration of the second unit dose, such as at day 120, at least 80%, or at least 85%, or at least 90% or at least 95% of the subject population are seropositive for all four dengue serotypes.
20. The composition for use of any one of items 11 to 14, wherein after the administration of the second unit dose, such as at day 270, at least 80%, or at least 85%, or at least 90% of the subject population are seropositive for all four dengue serotypes.
21. The composition for use of any one of items 16 to 20, wherein the composition provides a seropositivity rate, when it is administered to a subject population of at least 100 subjects in two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population comprises from 20% to 40% subjects who are seronegative to all dengue serotypes and from 60% to 80% subjects who are seropositive to at least one dengue serotype at base line, wherein at day 120 and/or day 270 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 10%-points and/or wherein at day 120 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 5%-points.
22. A method of inoculating a subject against virologically confirmable dengue disease in a subject comprising administering to the subject a tetravalent dengue virus composition including four dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4.
23. A method of inoculating a subject against virologically confirmable dengue disease consisting of administering to the subject a tetravalent dengue virus composition including four dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4.
24. The method of item 22 or 23, wherein the method does not comprise a determination of a previous dengue infection in the subject before the administration of the tetravalent dengue virus composition.
25. The method of any one of items 22 to 24, wherein the inoculation is safe irrespective of whether there is a determination that the subject had a previous dengue infection before the administration of the tetravalent dengue virus composition.
26. The method of any one of items 22 to 25 which is safe.
27. The method of any one of items 22 to 26 which is effective.
28. The method of any one of items 22 to 27, wherein the virus strains are live, attenuated dengue virus strains.
29. The method of any one of items 22 to 28, wherein the composition includes at least one chimeric dengue virus and optionally at least one non-chimeric dengue virus.

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30. The method of any one of items 22 to 29, wherein the composition includes a chimeric dengue serotype 2/1 strain and a dengue serotype 2 strain and a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain.
31. The method of any one of items 22 to 30, wherein the subject is seronegative to all dengue serotypes at base line and/or under 9 years of age, 4 to 5 years of age, 6 to 11 years of age or 12 to 16 years of age.
32. The method of any one of items 22 to 31, wherein the composition is administered by subcutaneous injection.
33. The method of any one of items 22 to 32 including consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least four 4 apart, optionally at about day 1 and at about day 90.
34. The method of any one of items 22 to 33, wherein the dengue serotypes 1 and 2 are present in similar amounts and/or make up less than about 10% of the total viral concentration.
35. The method of any one of items 22 to 34, wherein the dengue serotype 3 makes up at least about 10% of the total viral concentration.
36. The method of any one of items 22 to 35, wherein the dengue serotype 4 makes up at least about 70% of the total viral concentration.
37. The method of any one of items 22 to 36 wherein the dengue serotype 4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 and dengue serotype 2 each represent lower concentrations than the concentration of serotype 3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.
38. The method of any one of items 22 to 37, wherein the method is for preventing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).
39. The method of any one of items 22 to 38 wherein the subject or subject population is from a dengue endemic region.
40. The method of any one of items 22 to 38, wherein the subject or subject population is from a dengue non-endemic region.
41. The method of any one of items 22 to 40, wherein the subject or subject population is from Asia Pacific or Latin America.
42. The method of any one of items 22 to 41 having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, and optionally at least 4 weeks apart, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule.
43. The method of item 42, wherein the lower bound is more than 30%, is more than 40%, is more than 50%, is more

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- than 55%, is more than 60%, is more than 65%, is more than 70% or is more than 72%.
44. The method of any one of items 22 to 43 having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, and optionally at least 4 weeks apart, 30 days after the second administration until at least 12 months after the second administration.
45. The method of item 44, wherein the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 55%, is more than 60%, is more than 65%, is more than 70%, is more than 75% is more than 78%, is more than 79% or is about 80%.
46. The method of any one of items 22 to 45 having a combined vaccine efficacy against all four dengue serotypes in seronegative subjects with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule.
47. The method of item 46, wherein the lower bound is more than 30%, is more than 40%, is more than 50%, or is more than 55%.
48. The method of any one of items 22 to 47 having a combined vaccine efficacy against all four dengue serotypes in seronegative subjects of more than 30%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.
49. The method of item 48, wherein the combined vaccine efficacy against all four dengue serotypes in seronegative subjects is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 70%.
50. The method of any one of items 22 to 49 having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 4 to 5 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule.
51. The method of item 50, wherein the lower bound is more than 30%, is more than 40%, is more than 45%.
52. The method of any one of items 22 to 51 having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 4 to 5 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit

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- dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.
53. The method of item 52, wherein the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 70%.
54. The method of any one of items 22 to 53 having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 6 to 11 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule.
55. The method of item 54, wherein the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, or is more than 70%.
56. The method of any one of items 22 to 55 having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 6 to 11 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.
57. The method of item 56, wherein the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 75%, or is more than 80%.
58. The method of any one of items 26 to 57 having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 12 to 16 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule.
59. The method of item 58, wherein the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 68%.
60. The method of any one of items 26 to 59 having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 12 to 16 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.
61. The method of item 60, wherein the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 75%, or is more than 80%.

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62. The method of any one of items 22 to 61 having a vaccine efficacy against dengue serotype 1 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. 10
63. The method of item 62, wherein the lower bound is more than 30%, is more than 40%, or is more than 50%. 15
64. The method of any one of items 22 to 63 having a vaccine efficacy against dengue serotype 1 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. 20
65. The method of item 64, wherein the vaccine efficacy against dengue serotype 1 is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 70%. 25
66. The method of any one of items 22 to 65 having a vaccine efficacy against dengue serotype 2 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. 30
67. The method of item 66, wherein the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 90%. 35
68. The method of any one of items 22 to 67 having a vaccine efficacy against dengue serotype 2 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. 40
69. The method of item 68, wherein the vaccine efficacy against dengue serotype 2 is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 90%. 45
70. The method of any one of items 22 to 69 having a vaccine efficacy against dengue serotype 3 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. 50

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- administration schedule until at least 12 months after the second administration of the administration schedule.
71. The method of item 70, wherein the lower bound is more than 30%, is more than 40%. 5
72. The method of any one of items 22 to 71 having a vaccine efficacy against dengue serotype 3 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. 10
73. The method of item 72, wherein the vaccine efficacy against dengue serotype 3 is more than 40%, is more than 50%, is more than 55%, or is more than 60%. 15
74. The method of any one of items 22 to 73 having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. 20
75. The method of item 74, wherein the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 70%, or is more than 75%. 25
76. The method of any one of items 22 to 75 having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes of more than 30%, when measured against placebo in a subject population of at least 2,000 healthy subjects, healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. 30
77. The method of item 76, wherein the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 90%. 35
78. The method of any one of items 21 to 77 having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seropositive at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. 40
79. The method of item 78, wherein the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 70%, or is more than 80%. 45
80. The method of any one of items 22 to 79 having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four sero-

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types of more than 30%, when measured against placebo in a subject population of at least 2,000 healthy subjects, healthy subjects being seropositive at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, 5 30 days after the second administration until at least 12 months after the second administration.

81. The method of item 80, combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes is more than 40%, is more than 10 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 90%.

82. The method of any one of items 22 to 81 having a combined relative risk against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the upper bound is less than 0.75, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least 15 20 twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.

83. The method of item 82, wherein the upper bound is less than 0.70, is less than 0.65, is less than 0.60, is less than 0.55, is less than 0.50, is less than 0.45, is less than 0.40, is less than 0.35, is less than 0.30 or is less than 0.28.

84. The method of any one of items 22 to 83, wherein the combined relative risk against all four dengue serotypes is less than 0.70, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least 30 35 twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration.

85. The method of item 84, wherein the combined relative risk against all four serotypes is less than 0.65, is less than 0.60, is less than 0.55, is less than 0.50, is less than 0.45, is less than 0.40, is less than 0.35, is less than 0.30, is less than 0.25 or is less than 0.23.

86. The method of any one of items 22 to 85 having a combined vaccine efficacy against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 61.0%, or more than 65.0 or more than 70.0% or more than 72.0% when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 50 55 15,000 healthy subjects from endemic regions irrespective of serostatus at baseline and being selected from the group consisting of 4 to 16 year old subjects at the time of randomization, wherein said unit dose or said placebo is administered at least twice within 6 months or less, about 30 days after the last administration of the administration schedule until at least 12 or 13 months after the last administration of the administration schedule.

87. The method of any one of items 22 to 86 having a combined vaccine efficacy against all four serotypes of more than 66%, or of more than 70%, or of more than 75%, or of more than 77%, or of more than 80.0%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects from endemic regions irrespective of serostatus at baseline and being selected from the group consisting of 4 to 16 year old subjects at the time of randomization, wherein said unit 60 65

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dose or said placebo is administered at least twice within 6 months or less, about 30 days after the last administration of the administration schedule until at least 12 months or 13 month after the last administration of the administration schedule.

88. The method of any one of items 22 to 87, wherein said unit dose or said placebo is administered at day 1 and day 90.

89. The method of any one of items 22 to 88 having a relative risk for virologically confirmed dengue with hospitalization which is 1 or less, or 0.8 or less, or 0.6 or less, when measured against placebo in a subject population of at least 1,000 healthy subjects, or at least 5,000 healthy subjects, or at least 10,000 healthy subjects irrespective of serostatus at baseline and in age groups from 4 to 16 years, in particular in subjects 4 to 5 years of age at the time of randomization.

90. The method of any one of items 22 to 89, wherein the occurrence of vaccine related serious adverse events is less than 0.1%.

91. The method of any one of items 22 to 90, wherein the occurrence of vaccine related unsolicited adverse events occurring within 4 weeks of administration is less than 2%.

92. The method of any one of items 22 to 91, wherein the occurrence of vaccine related solicited adverse events occurring within 2 weeks of administration is less than 35%.

93. The method of any one of items 22 to 92, wherein the occurrence of solicited local reactions occurring within 1 weeks of administration is less than 40%.

94. The method of any one of items 22 to 93, wherein the unit dose upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent

- (i) dengue serotype 1 has a concentration of 3.3 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,
- (ii) dengue serotype 2 has a concentration of 2.7 log 10 pfu/0.5 mL to 4.9 log 10 pfu/0.5 mL,
- (iii) dengue serotype 3 has a concentration of 4.0 log 10 pfu/0.5 mL to 5.7 log 10 pfu/0.5 mL, and
- (iv) dengue serotype 4 has a concentration of 4.5 log 10 pfu/0.5 mL to 6.2 log 10 pfu/0.5 mL,

and optionally comprises about 15% (w/v) α,α -trehalose dihydrate, about 1% (w/v) poloxamer 407, about 0.1% (w/v) human serum albumin, and about 100 mM sodium chloride when measured in 0.5 mL.

95. A reconstituted unit dose of a dengue vaccine composition for use in a method of preventing virologically confirmable dengue disease in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, wherein the dengue vaccine composition is a tetravalent dengue virus composition including four dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, optionally wherein the dengue virus strains are live, attenuated, and wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent

- (i) dengue serotype 1 has a concentration of at least 3.3 log 10 pfu/0.5 mL,
- (ii) dengue serotype 2 has a concentration of at least 2.7 log 10 pfu/0.5 mL,
- (iii) dengue serotype 3 has a concentration of at least 4.0 log 10 pfu/0.5 mL, and

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- (iv) dengue serotype 4 has a concentration of at least 4.5 log 10 pfu/0.5 mL.
96. The unit dose for use of item 95, wherein the subject is under 9 years of age and/or when the serostatus of the subject is unknown or seronegative.
97. The unit dose for use of item 95 or 96, which is effective.
98. The unit dose for use of any one of items 95 to 97, which is effective against all four dengue serotypes.
99. The unit dose for use of any one of items 95 to 98, which is safe.
100. The unit dose for use of any one of items 95 to 99, wherein the unit dose includes at least one chimeric dengue virus.
101. The unit dose for use of any one of items 95 to 100, wherein the unit dose includes at least one non-chimeric dengue virus and at least one chimeric dengue virus.
102. The unit dose for use of any one of items 95 to 101, wherein the subject is seronegative to all dengue serotypes at baseline and/or is under 9 years of age.
103. The unit dose for use of any one of items 95 to 102, wherein the subject is 4 to 5 years of age or 6 to 11 years of age or 12 to 16 years of age.
104. The unit dose for use of any one of items 95 to 103, wherein the method does not comprise a determination of a previous dengue infection in the subject before the administration of the first unit dose of the tetravalent dengue virus composition.
105. The unit dose for use of any one of items 95 to 104, wherein the dengue serotype 4 represents the highest concentration in the composition of all four serotypes, optionally with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 represents the second highest concentration in the composition of all four serotypes with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 and dengue serotype 2 each represent lower concentrations than the concentration of serotype 3 and together represent less than about 10% of the total concentration in pfu/0.5 mL and/or which are within 5%-points of each other.
106. The unit dose for use of any one of items 95 to 105, wherein the dengue serotype 1 is a chimeric dengue serotype 2/1 strain, the dengue serotype 2 is a non-chimeric dengue serotype 2 strain, the dengue serotype 3 is a chimeric dengue serotype 2/3 strain and the dengue serotype 4 is a chimeric dengue serotype 2/4 strain.
107. The unit dose for use of any one of items 95 to 106, wherein the dengue serotype 1 has the amino acid sequence of SEQ ID NO. 2, the dengue serotype 2 has the amino acid sequence of SEQ ID NO. 4, the dengue serotype 3 has the amino acid sequence of SEQ ID NO. 6, and the dengue serotype 4 has the amino acid sequence of SEQ ID NO. 8.
108. The unit dose for use of any one of items 95 to 107, wherein the unit dose further comprises from about 10 w/v to about 20% w/v α,α -trehalose dihydrate or an equimolar amount of other forms of α,α -trehalose, from about 0.5% w/v to about 1.5% w/v poloxamer 407, from about 0.05% w/v to about 2% w/v human serum albumin, and from about 70 mM to 140 mM sodium chloride when measured in 0.5 mL.
109. The unit dose for use of any one of items 95 to 108, wherein the unit dose further comprises about 15 (w/v) α,α -trehalose dihydrate, about 1% (w/v) poloxamer 407, about 0.1% (w/v) human serum albumin, and about 100 mM sodium chloride when measured in 0.5 mL.

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110. The unit dose for use of any one of items 95 to 109, wherein the method is for preventing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).
111. The unit dose for use of any one of items 95 to 110, wherein the subject is from a dengue endemic region.
112. The unit dose for use of any one of items 95 to 111, wherein the subject is from a dengue non-endemic region.
113. The unit dose for use of any one of items 95 to 112, wherein the subject is from Asia Pacific or Latin America.
114. The unit dose for use of any one items of 95 to 113, wherein the unit dose provides a seropositivity rate when it is administered to a subject population of at least 50 subjects in two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population are seronegative to all dengue serotypes at baseline.
115. The unit dose for use of item 114, wherein at least one month after administration of the first unit dose, such as at day 30, at least 80% of the subject population are seropositive for all four dengue serotypes.
116. The unit dose for use of item 114 or 115, wherein before or at the time of the administration of the second unit dose, such as at day 90, at least 80% of the subject population are seropositive for all four dengue serotypes.
117. The unit dose for use of any one of items 114 to 116, wherein after the administration of the second unit dose, such as at day 120, at least 80%, or at least 85%, or at least 90% or at least 95% of the subject population are seropositive for all four dengue serotypes.
118. The unit dose for use of any one of items 114 to 117, wherein after the administration of the second unit dose, such as at day 270, at least 80%, or at least 85%, or at least 90% of the subject population are seropositive for all four dengue serotypes.
119. The unit dose for use of any one of items 114 to 118, wherein the unit dose provides a seropositivity rate, when it is administered to a subject population of at least 100 subjects in two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population comprises from 20% to 40% subjects who are seronegative to all dengue serotypes and from 60% to 80% subjects who are seropositive to at least one dengue serotype at base line, wherein at day 120 and/or day 270 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 10%-points and/or wherein at day 120 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 5%-points.
120. The unit dose for use of any one of items 95 to 119, wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent
- (i) dengue serotype 1 has a concentration of 3.3 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,
 - (ii) dengue serotype 2 has a concentration of 2.7 log 10 pfu/0.5 mL to 4.9 log 10 pfu/0.5 mL,
 - (iii) dengue serotype 3 has a concentration of 4.0 log 10 pfu/0.5 mL to 5.7 log 10 pfu/0.5 mL, and
 - (iv) dengue serotype 4 has a concentration of 4.5 log 10 pfu/0.5 mL to 6.2 log 10 pfu/0.5 mL.
121. The unit dose for use of any one of items 95 to 120, wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent:

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- (i) dengue serotype 1 has a concentration of $3.3 \log 10$ pfu/0.5 mL to $5.0 \log 10$ pfu/0.5 mL,
 - (ii) dengue serotype 2 has a concentration of $2.7 \log 10$ pfu/0.5 mL to $4.9 \log 10$ pfu/0.5 mL,

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- (iii) dengue serotype 3 has a concentration of $4.0 \log_{10}$ pfu/0.5 mL to $5.7 \log_{10}$ pfu/0.5 mL, and
 - (iv) dengue serotype 4 has a concentration of $4.5 \log_{10}$ pfu/0.5 mL to $5.5 \log_{10}$ pfu/0.5 mL.

SEQUENCE LISTING

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<210> SEQ ID NO 1

<211> LENGTH: 10723

<212> TYPE: DNA

<213> ORGANISM: chimeric dengue serotype 2/1 (MVS)

<400> SEQUENCE: 1

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<210> SEQ ID NO 2

<211> LENGTH: 3391

<212> TYPE: PRT

<213> ORGANISM: chimeric dengue serotype 2/1 (MVS)

<400> SEQUENCE: 2

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Lys	Arg	Glu	Arg	Asn	Arg	Val	Ser	Thr	Val	Gln	Gln	Leu	Thr	Lys	Arg
20															30

Phe	Ser	Leu	Gly	Met	Leu	Gln	Gly	Arg	Gly	Pro	Leu	Lys	Leu	Phe	Met
35															45

Ala	Leu	Val	Ala	Phe	Leu	Arg	Phe	Leu	Thr	Ile	Pro	Pro	Thr	Ala	Gly
50															60

Ile	Leu	Lys	Arg	Trp	Gly	Thr	Ile	Lys	Lys	Ser	Lys	Ala	Ile	Asn	Val
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65	70	75	80
Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn			
85	90	95	
Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val			
100	105	110	
Met Ala Phe His Leu Thr Thr Arg Gly Gly Glu Pro His Met Ile Val			
115	120	125	
Ser Lys Gln Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ser Ala Gly			
130	135	140	
Val Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Leu Cys Glu			
145	150	155	160
Asp Thr Met Thr Tyr Lys Cys Pro Arg Ile Thr Glu Ala Glu Pro Asp			
165	170	175	
Asp Val Asp Cys Trp Cys Asn Ala Thr Asp Thr Trp Val Thr Tyr Gly			
180	185	190	
Thr Cys Ser Gln Thr Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala			
195	200	205	
Leu Ala Pro His Val Gly Leu Gly Leu Glu Thr Arg Ala Glu Thr Trp			
210	215	220	
Met Ser Ser Glu Gly Ala Trp Lys Gln Ile Gln Lys Val Glu Thr Trp			
225	230	235	240
Ala Leu Arg His Pro Gly Phe Thr Val Ile Ala Leu Phe Leu Ala His			
245	250	255	
Ala Ile Gly Thr Ser Ile Thr Gln Lys Gly Ile Ile Phe Ile Leu Leu			
260	265	270	
Met Leu Val Thr Pro Ser Met Ala Met Arg Cys Val Gly Ile Gly Asn			
275	280	285	
Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val			
290	295	300	
Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr			
305	310	315	320
Leu Asp Ile Glu Leu Leu Lys Thr Glu Val Thr Asn Pro Ala Val Leu			
325	330	335	
Arg Lys Leu Cys Ile Glu Ala Lys Ile Ser Asn Thr Thr Asp Ser			
340	345	350	
Arg Cys Pro Thr Gln Gly Glu Ala Thr Leu Val Glu Glu Gln Asp Ala			
355	360	365	
Asn Phe Val Cys Arg Arg Thr Phe Val Asp Arg Gly Trp Gly Asn Gly			
370	375	380	
Cys Gly Leu Phe Gly Lys Gly Ser Leu Ile Thr Cys Ala Lys Phe Lys			
385	390	395	400
Cys Val Thr Lys Leu Glu Gly Lys Ile Val Gln Tyr Glu Asn Leu Lys			
405	410	415	
Tyr Ser Val Ile Val Thr Val His Thr Gly Asp Gln His Gln Val Gly			
420	425	430	
Asn Glu Thr Thr Glu His Gly Thr Thr Ala Thr Ile Thr Pro Gln Ala			
435	440	445	
Pro Thr Ser Glu Ile Gln Leu Thr Asp Tyr Gly Thr Leu Thr Leu Asp			
450	455	460	
Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Thr			
465	470	475	480
Met Lys Glu Arg Ser Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu			
485	490	495	

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Pro Leu Pro Trp Thr Ser Gly Ala Ser Thr Ser Gln Glu Thr Trp Asn
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 Arg Gln Asp Leu Leu Val Thr Phe Lys Thr Ala His Ala Lys Lys Gln
 515 520 525
 Glu Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
 530 535 540
 Thr Gly Ala Thr Glu Ile Gln Thr Ser Gly Thr Thr Thr Ile Phe Ala
 545 550 555 560
 Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Thr Leu Lys Gly
 565 570 575
 Met Ser Tyr Val Met Cys Thr Gly Ser Phe Lys Leu Glu Lys Glu Val
 580 585 590
 Ala Glu Thr Gln His Gly Thr Val Leu Val Gln Val Lys Tyr Glu Gly
 595 600 605
 Thr Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr Gln Asp Glu Lys Gly
 610 615 620
 Ala Thr Gln Asn Gly Arg Leu Ile Thr Ala Asn Pro Ile Val Thr Asp
 625 630 635 640
 Lys Glu Lys Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser
 645 650 655
 Tyr Ile Val Val Gly Ala Gly Glu Lys Ala Leu Lys Leu Ser Trp Phe
 660 665 670
 Lys Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly
 675 680 685
 Ala Arg Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
 690 695 700
 Ile Gly Gly Val Phe Thr Ser Met Gly Lys Leu Val His Gln Val Phe
 705 710 715 720
 Gly Thr Ala Tyr Gly Val Leu Phe Ser Gly Val Ser Trp Thr Met Lys
 725 730 735
 Ile Gly Ile Gly Ile Leu Leu Thr Trp Leu Gly Leu Asn Ser Arg Asn
 740 745 750
 Thr Ser Leu Ser Met Met Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr
 755 760 765
 Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
 770 775 780
 Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
 785 790 795 800
 His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
 805 810 815
 Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
 820 825 830
 Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
 835 840 845
 Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
 850 855 860
 Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
 865 870 875 880
 Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
 885 890 895
 Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
 900 905 910

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Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
 915 920 925
 Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
 930 935 940
 Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
 945 950 955 960
 Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
 965 970 975
 Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
 980 985 990
 Ile Glu Val Lys Asn Cys His Trp Pro Lys Ser His Thr Leu Trp Ser
 995 1000 1005
 Asn Gly Val Leu Glu Ser Glu Met Ile Ile Pro Lys Asn Leu Ala
 1010 1015 1020
 Gly Pro Val Ser Gln His Asn Tyr Arg Pro Gly Tyr His Thr Gln
 1025 1030 1035
 Ile Thr Gly Pro Trp His Leu Gly Lys Leu Glu Met Asp Phe Asp
 1040 1045 1050
 Phe Cys Asp Gly Thr Thr Val Val Val Thr Glu Asp Cys Gly Asn
 1055 1060 1065
 Arg Gly Pro Ser Leu Arg Thr Thr Thr Ala Ser Gly Lys Leu Ile
 1070 1075 1080
 Thr Glu Trp Cys Cys Arg Ser Cys Thr Leu Pro Pro Leu Arg Tyr
 1085 1090 1095
 Arg Gly Glu Asp Gly Cys Trp Tyr Gly Met Glu Ile Arg Pro Leu
 1100 1105 1110
 Lys Glu Lys Glu Glu Asn Leu Val Asn Ser Leu Val Thr Ala Gly
 1115 1120 1125
 His Gly Gln Val Asp Asn Phe Ser Leu Gly Val Leu Gly Met Ala
 1130 1135 1140
 Leu Phe Leu Glu Glu Met Leu Arg Thr Arg Val Gly Thr Lys His
 1145 1150 1155
 Ala Ile Leu Leu Val Ala Val Ser Phe Val Thr Leu Ile Thr Gly
 1160 1165 1170
 Asn Met Ser Phe Arg Asp Leu Gly Arg Val Met Val Met Val Gly
 1175 1180 1185
 Ala Thr Met Thr Asp Asp Ile Gly Met Gly Val Thr Tyr Leu Ala
 1190 1195 1200
 Leu Leu Ala Ala Phe Lys Val Arg Pro Thr Phe Ala Ala Gly Leu
 1205 1210 1215
 Leu Leu Arg Lys Leu Thr Ser Lys Glu Leu Met Met Thr Thr Ile
 1220 1225 1230
 Gly Ile Val Leu Leu Ser Gln Ser Thr Leu Pro Glu Thr Ile Leu
 1235 1240 1245
 Glu Leu Thr Asp Ala Leu Ala Leu Gly Met Met Val Leu Lys Met
 1250 1255 1260
 Val Arg Asn Met Glu Lys Tyr Gln Leu Ala Val Thr Ile Met Ala
 1265 1270 1275
 Ile Leu Cys Val Pro Asn Ala Val Ile Leu Gln Asn Ala Trp Lys
 1280 1285 1290
 Val Ser Cys Thr Ile Leu Ala Val Val Ser Val Ser Pro Leu Phe
 1295 1300 1305
 Leu Thr Ser Ser Gln Gln Lys Thr Asp Trp Ile Pro Leu Ala Leu

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1310	1315	1320
Thr Ile Lys Gly Leu Asn Pro	Thr Ala Ile Phe Leu	Thr Thr Leu
1325	1330	1335
Ser Arg Thr Ser Lys Lys Arg	Ser Trp Pro Leu Asn	Glu Ala Ile
1340	1345	1350
Met Ala Val Gly Met Val Ser	Ile Leu Ala Ser Ser	Leu Leu Lys
1355	1360	1365
Asn Asp Ile Pro Met Thr Gly	Pro Leu Val Ala Gly	Gly Leu Leu
1370	1375	1380
Thr Val Cys Tyr Val Leu Thr	Gly Arg Ser Ala Asp	Leu Glu Leu
1385	1390	1395
Glu Arg Ala Ala Asp Val Lys	Trp Glu Asp Gln Ala	Glu Ile Ser
1400	1405	1410
Gly Ser Ser Pro Ile Leu Ser	Ile Thr Ile Ser Glu	Asp Gly Ser
1415	1420	1425
Met Ser Ile Lys Asn Glu Glu	Glu Asp Gln Thr	Leu Thr Ile Leu
1430	1435	1440
Ile Arg Thr Gly Leu Leu Val	Ile Ser Gly Leu Phe	Pro Val Ser
1445	1450	1455
Ile Pro Ile Thr Ala Ala Ala	Trp Tyr Leu Trp Glu	Val Lys Lys
1460	1465	1470
Gln Arg Ala Gly Val Leu Trp	Asp Val Pro Ser Pro	Pro Pro Met
1475	1480	1485
Gly Lys Ala Glu Leu Glu Asp	Gly Ala Tyr Arg Ile	Lys Gln Lys
1490	1495	1500
Gly Ile Leu Gly Tyr Ser Gln	Ile Gly Ala Gly Val	Tyr Lys Glu
1505	1510	1515
Gly Thr Phe His Thr Met Trp	His Val Thr Arg Gly	Ala Val Leu
1520	1525	1530
Met His Lys Gly Lys Arg Ile	Glu Pro Ser Trp Ala	Asp Val Lys
1535	1540	1545
Lys Asp Leu Ile Ser Tyr Gly	Gly Gly Trp Lys Leu	Glu Gly Glu
1550	1555	1560
Trp Lys Glu Gly Glu Val	Gln Val Leu Ala Leu	Glu Pro Gly
1565	1570	1575
Lys Asn Pro Arg Ala Val Gln	Thr Lys Pro Gly Leu	Phe Lys Thr
1580	1585	1590
Asn Ala Gly Thr Ile Gly Ala	Val Ser Leu Asp Phe	Ser Pro Gly
1595	1600	1605
Thr Ser Gly Ser Pro Ile Ile	Asp Lys Lys Gly Lys	Val Val Gly
1610	1615	1620
Leu Tyr Gly Asn Gly Val Val	Thr Arg Ser Gly Ala	Tyr Val Ser
1625	1630	1635
Ala Ile Ala Gln Thr Glu Lys	Ser Ile Glu Asp Asn	Pro Glu Ile
1640	1645	1650
Glu Asp Asp Ile Phe Arg Lys	Arg Arg Leu Thr Ile	Met Asp Leu
1655	1660	1665
His Pro Gly Ala Gly Lys Thr	Lys Arg Tyr Leu Pro	Ala Ile Val
1670	1675	1680
Arg Glu Ala Ile Lys Arg Gly	Leu Arg Thr Leu Ile	Leu Ala Pro
1685	1690	1695
Thr Arg Val Val Ala Ala Glu	Met Glu Glu Ala Leu	Arg Gly Leu
1700	1705	1710

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Pro Ile Arg Tyr Gln Thr Pro Ala Ile Arg Ala Val His Thr Gly
 1715 1720 1725
 Arg Glu Ile Val Asp Leu Met Cys His Ala Thr Phe Thr Met Arg
 1730 1735 1740
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 1745 1750 1755
 Asp Glu Ala His Phe Thr Asp Pro Ala Ser Ile Ala Ala Arg Gly
 1760 1765 1770
 Tyr Ile Ser Thr Arg Val Glu Met Gly Glu Ala Ala Gly Ile Phe
 1775 1780 1785
 Met Thr Ala Thr Pro Pro Gly Ser Arg Asp Pro Phe Pro Gln Ser
 1790 1795 1800
 Asn Ala Pro Ile Ile Asp Glu Glu Arg Glu Ile Pro Glu Arg Ser
 1805 1810 1815
 Trp Asn Ser Gly His Glu Trp Val Thr Asp Phe Lys Gly Lys Thr
 1820 1825 1830
 Val Trp Phe Val Pro Ser Ile Lys Ala Gly Asn Asp Ile Ala Ala
 1835 1840 1845
 Cys Leu Arg Lys Asn Gly Lys Lys Val Ile Gln Leu Ser Arg Lys
 1850 1855 1860
 Thr Phe Asp Ser Glu Tyr Val Lys Thr Arg Thr Asn Asp Trp Asp
 1865 1870 1875
 Phe Val Val Thr Thr Asp Ile Ser Glu Met Gly Ala Asn Phe Lys
 1880 1885 1890
 Ala Glu Arg Val Ile Asp Pro Arg Arg Cys Met Lys Pro Val Ile
 1895 1900 1905
 Leu Thr Asp Gly Glu Glu Arg Val Ile Leu Ala Gly Pro Met Pro
 1910 1915 1920
 Val Thr His Ser Ser Ala Ala Gln Arg Arg Gly Arg Ile Gly Arg
 1925 1930 1935
 Asn Pro Lys Asn Glu Asn Asp Gln Tyr Ile Tyr Met Gly Glu Pro
 1940 1945 1950
 Leu Glu Asn Asp Glu Asp Cys Ala His Trp Lys Glu Ala Lys Met
 1955 1960 1965
 Leu Leu Asp Asn Ile Asn Thr Pro Glu Gly Ile Ile Pro Ser Met
 1970 1975 1980
 Phe Glu Pro Glu Arg Glu Lys Val Asp Ala Ile Asp Gly Glu Tyr
 1985 1990 1995
 Arg Leu Arg Gly Glu Ala Arg Lys Thr Phe Val Asp Leu Met Arg
 2000 2005 2010
 Arg Gly Asp Leu Pro Val Trp Leu Ala Tyr Arg Val Ala Ala Glu
 2015 2020 2025
 Gly Ile Asn Tyr Ala Asp Arg Arg Trp Cys Phe Asp Gly Val Lys
 2030 2035 2040
 Asn Asn Gln Ile Leu Glu Glu Asn Val Glu Val Glu Ile Trp Thr
 2045 2050 2055
 Lys Glu Gly Glu Arg Lys Lys Leu Lys Pro Arg Trp Leu Asp Ala
 2060 2065 2070
 Arg Ile Tyr Ser Asp Pro Leu Ala Leu Lys Glu Phe Lys Glu Phe
 2075 2080 2085
 Ala Ala Gly Arg Lys Ser Leu Thr Leu Asn Leu Ile Thr Glu Met
 2090 2095 2100

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Gly Arg Leu Pro Thr Phe Met Thr Gln Lys Ala Arg Asp Ala Leu
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Asp Asn Leu Ala Val Leu His Thr Ala Glu Ala Gly Gly Arg Ala
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Tyr Asn His Ala Leu Ser Glu Leu Pro Glu Thr Leu Glu Thr Leu
2135 2140 2145

Leu Leu Leu Thr Leu Leu Ala Thr Val Thr Gly Gly Ile Phe Leu
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Phe Leu Met Ser Ala Arg Gly Ile Gly Lys Met Thr Leu Gly Met
2165 2170 2175

Cys Cys Ile Ile Thr Ala Ser Ile Leu Leu Trp Tyr Ala Gln Ile
2180 2185 2190

Gln Pro His Trp Ile Ala Ala Ser Ile Ile Leu Glu Phe Phe Leu
2195 2200 2205

Ile Val Leu Leu Ile Pro Glu Pro Glu Lys Gln Arg Thr Pro Gln
2210 2215 2220

Asp Asn Gln Leu Thr Tyr Val Val Ile Ala Ile Leu Thr Val Val
2225 2230 2235

Ala Ala Thr Met Ala Asn Glu Met Gly Phe Leu Glu Lys Thr Lys
2240 2245 2250

Lys Asp Leu Gly Leu Gly Ser Ile Ala Thr Gln Gln Pro Glu Ser
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Asn Ile Leu Asp Ile Asp Leu Arg Pro Ala Ser Ala Trp Thr Leu
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Tyr Ala Val Ala Thr Thr Phe Val Thr Pro Met Leu Arg His Ser
2285 2290 2295

Ile Glu Asn Ser Ser Val Asn Val Ser Leu Thr Ala Ile Ala Asn
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Gln Ala Thr Val Leu Met Gly Leu Gly Lys Gly Trp Pro Leu Ser
2315 2320 2325

Lys Met Asp Ile Gly Val Pro Leu Leu Ala Ile Gly Cys Tyr Ser
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Gln Val Asn Pro Ile Thr Leu Thr Ala Ala Leu Phe Leu Leu Val
2345 2350 2355

Ala His Tyr Ala Ile Ile Gly Pro Gly Leu Gln Ala Lys Ala Thr
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Arg Glu Ala Gln Lys Arg Ala Ala Ala Gly Ile Met Lys Asn Pro
2375 2380 2385

Thr Val Asp Gly Ile Thr Val Ile Asp Leu Asp Pro Ile Pro Tyr
2390 2395 2400

Asp Pro Lys Phe Glu Lys Gln Leu Gly Gln Val Met Leu Leu Val
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Leu Cys Val Thr Gln Val Leu Met Met Arg Thr Thr Trp Ala Leu
2420 2425 2430

Cys Glu Ala Leu Thr Leu Ala Thr Gly Pro Ile Ser Thr Leu Trp
2435 2440 2445

Glu Gly Asn Pro Gly Arg Phe Trp Asn Thr Thr Ile Ala Val Ser
2450 2455 2460

Met Ala Asn Ile Phe Arg Gly Ser Tyr Leu Ala Gly Ala Gly Leu
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Leu Phe Ser Ile Met Lys Asn Thr Thr Asn Thr Arg Arg Gly Thr
2480 2485 2490

Gly Asn Ile Gly Glu Thr Leu Gly Glu Lys Trp Lys Ser Arg Leu

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Ile Gln	Glu Val Asp Arg Thr	Leu Ala Lys Glu Gly Ile Lys Arg
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Gly Glu	Thr Asp His His Ala	Val Ser Arg Gly Ser Ala Lys Leu
2540	2545	2550
Arg Trp	Phe Val Glu Arg Asn	Met Val Thr Pro Glu Gly Lys Val
2555	2560	2565
Val Asp	Leu Gly Cys Gly Arg	Gly Gly Trp Ser Tyr Tyr Cys Gly
2570	2575	2580
Gly Leu	Lys Asn Val Arg Glu	Val Lys Gly Leu Thr Lys Gly Gly
2585	2590	2595
Pro Gly	His Glu Glu Pro Ile	Pro Met Ser Thr Tyr Gly Trp Asn
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Leu Val	Arg Leu Gln Ser Gly	Val Asp Val Phe Phe Ile Pro Pro
2615	2620	2625
Glu Lys	Cys Asp Thr Leu Leu	Cys Asp Ile Gly Glu Ser Ser Pro
2630	2635	2640
Asn Pro	Thr Val Glu Ala Gly	Arg Thr Leu Arg Val Leu Asn Leu
2645	2650	2655
Val Glu	Asn Trp Leu Asn Asn	Asn Thr Gln Phe Cys Ile Lys Val
2660	2665	2670
Leu Asn	Pro Tyr Met Pro Ser	Val Ile Glu Lys Met Glu Ala Leu
2675	2680	2685
Gln Arg	Lys Tyr Gly Gly Ala	Leu Val Arg Asn Pro Leu Ser Arg
2690	2695	2700
Asn Ser	Thr His Glu Met Tyr	Trp Val Ser Asn Ala Ser Gly Asn
2705	2710	2715
Ile Val	Ser Ser Val Asn Met	Ile Ser Arg Met Leu Ile Asn Arg
2720	2725	2730
Phe Thr	Met Arg Tyr Lys Lys	Ala Thr Tyr Glu Pro Asp Val Asp
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Leu Gly	Ser Gly Thr Arg Asn	Ile Gly Ile Glu Ser Glu Ile Pro
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Asn Leu	Asp Ile Ile Gly Lys	Arg Ile Glu Lys Ile Lys Gln Glu
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His Glu	Thr Ser Trp His Tyr	Asp Gln Asp His Pro Tyr Lys Thr
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Trp Ala	Tyr His Gly Ser Tyr	Glu Thr Lys Gln Thr Gly Ser Ala
2795	2800	2805
Ser Ser	Met Val Asn Gly Val	Val Arg Leu Leu Thr Lys Pro Trp
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Pro Phe	Gly Gln Gln Arg Val	Phe Lys Glu Lys Val Asp Thr Arg
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Ala Glu	Trp Leu Trp Lys Glu	Leu Gly Lys Lys Lys Thr Pro Arg
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Met Cys	Thr Arg Glu Glu Phe	Thr Arg Lys Val Arg Ser Asn Ala
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 2945 2950 2955
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 Gln Trp Glu Pro Ser Arg Gly Trp Asn Asp Trp Thr Gln Val Pro
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 Phe Cys Ser His His Phe His Glu Leu Ile Met Lys Asp Gly Arg
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 Val Leu Val Val Pro Cys Arg Asn Gln Asp Glu Leu Ile Gly Arg
 3215 3220 3225
 Ala Arg Ile Ser Gln Gly Ala Gly Trp Ser Leu Arg Glu Thr Ala
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 Cys Leu Gly Lys Ser Tyr Ala Gln Met Trp Ser Leu Met Tyr Phe
 3245 3250 3255
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 3275 3280 3285

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3305					3310						3315			
Thr	Pro	Val	Glu	Ser	Trp	Glu	Glu	Ile	Pro	Tyr	Leu	Gly	Lys	Arg
3320				3325							3330			
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<210> SEQ ID NO 3

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<212> TYPE: DNA

<213> ORGANISM: dengue serotype 2 (MVS)

<400> SEQUENCE: 3

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tttgcataa	aggttctcaa	cccatatatg	ccctcagtca	tagaaaaat	ggaagcacta	8160
caaaggaaat	atggaggagc	cttagtgagg	aatccactct	cacgaaactc	cacacatgag	8220
atgtactggg	tatccaatgc	ttccggaaac	atagtgtcat	cagtgaacat	gattcaagg	8280
atgttgatca	acagatttac	aatgagatac	aagaaagcca	cttacgagcc	ggatgttgac	8340
ctcggaaagcg	gaacccgtaa	catcgggatt	gaaagtgaga	taccaaacc	agatataatt	8400
ggggaaaagaa	tagaaaaat	aaagcaagag	catgaaacat	catggacta	tgaccaagac	8460
cacccatata	aaacgtggc	ataccatgt	agctatgaaa	aaaaacagac	tggatcagca	8520
tcatccatgg	tcaacggagt	ggtcaggctg	ctgacaaaac	cttggacgt	cgtccccatg	8580
gtgacacaga	tggcaatgac	agacacgact	ccatttggac	aacagcgcgt	ttttaaagag	8640

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aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca	8700
gcagagtggc tttggaaaga attagggaaag aaaaagacac ccaggatgtg caccagagaa	8760
gaattcacaa gaaaggtgag aagcaatgca gccttgggg ccgtattcac tgatgagaac	8820
aagtggaaat cggcacgtga ggctgttcaa gatacgatgt ttggagct gggtgacaag	8880
gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatggaaaa	8940
agagagaaga agcttagggaa attcggcaag gcaaaaggca gcagagccat atggatcatg	9000
tggcttggag cacgttctt agagtttcaa gcccttaggt tcttaatga agatcaactgg	9060
ttctccagag agaactccct gagttggatg gaaggagaag ggctgcacaa gctagttac	9120
attctaagag acgtgagcaa gaaagaggaa ggagcaatgt atgccatgtg caccgcagga	9180
tgggatacaa gaatcacact agaagaccta aaaaatgaaag aaatggtaac aaaccacatg	9240
gaaggagaac acaagaaaact agccgaggcc atttcaaac taacgtacca aaacaaggatg	9300
gtgcgtgtgc aaagaccaac accaagaggg acagtaatgg acatcatatc gagaagagac	9360
caaagaggtt gtggacaagt tggcacctat ggactcaata ctccaccaa tatggaaagcc	9420
caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaaacatc	9480
acagaagaaa tcgctgtca aaactggta gcaagagtg ggccgcgaaag gttatcaaga	9540
atggccatca gtggagatga ttgtgttgc aaaccttttag atgacaggtt cgcaagcgct	9600
ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca	9660
agaggatgga atgattggac acaagtgcctt ttctgttcac accatccatc tgatgttac	9720
atgaaagacg gtcgegtact cgttgcattca tgtagaaacc aagatgaact gattggcaga	9780
gcccgaatct cccaaggagc aggggtgtct ttgcgggaga cggcctgtt ggggaagtct	9840
tacgccccaa tgtggagctt gatgtacttc cacagacgac acctcaggct ggccggaaat	9900
gttatgttgc cggcgttacc atcacattgg gttccaaacaa gtcgaacaac ctggccata	9960
catgctaaac atgaatggat gacaacggaa gacatgtga cagtctggaa cagggtgtgg	10020
attcaagaaa acccatggat ggaagacaaa actccagttt aatcatggaa ggaaatccca	10080
tacttgggaa aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcaggccc	10140
acctggccaa agaacatcca agcagcaata aatcaagtta gatccctttag aggcaatgaa	10200
gaatacacatc attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga	10260
gttctgtgtt agaaagcaaa actaacatgt aacaaggctt gaaatgttggatc cggattaagc	10320
catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca	10380
ggccatcata aatgccatag ctttagttaaa ctatgcagcc tgcgttccat cctgagaagg	10440
tgtaaaaat cccggaggcc acaaccatg gaagctgtac gcatggcgta gtggacttagc	10500
ggtttagagga gaccctccc ttacaaatcg cagcaacaat gggggcccaa ggccgagatga	10560
agctgttagtc tcgctggaaag gacttagaggt tagaggagac cccccccaaa caaaaaacag	10620
catattgacg ctggaaaga ccagagatcc tgctgttccat tccaggcaca	10680
gaacgcaga aatggaaatg gtgctgttgc atcaacaggat tot	10723

<210> SEQ ID NO 4
<211> LENGTH: 3391
<212> TYPE: PRT
<213> ORGANISM: dengue serotype 2 (MVS)

<400> SEQUENCE: 4

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Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
 1 5 10 15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
 20 25 30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
 35 40 45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
 50 55 60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Ser Lys Ala Ile Asn Val
 65 70 75 80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
 85 90 95

Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
 100 105 110

Met Ala Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val
 115 120 125

Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Val Gly
 130 135 140

Val Asn Met Cys Thr Leu Met Ala Met Asp Leu Gly Glu Leu Cys Glu
 145 150 155 160

Asp Thr Ile Thr Tyr Glu Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu
 165 170 175

Asp Ile Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly
 180 185 190

Thr Cys Thr Thr Met Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala
 195 200 205

Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp
 210 215 220

Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp
 225 230 235 240

Ile Leu Arg His Pro Gly Phe Thr Met Met Ala Ala Ile Leu Ala Tyr
 245 250 255

Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu
 260 265 270

Thr Ala Val Thr Pro Ser Met Thr Met Arg Cys Ile Gly Met Ser Asn
 275 280 285

Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val
 290 295 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
 305 310 315 320

Leu Asp Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu
 325 330 335

Arg Lys Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Glu Ser
 340 345 350

Arg Cys Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys
 355 360 365

Arg Phe Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly
 370 375 380

Cys Gly Leu Phe Gly Lys Gly Ile Val Thr Cys Ala Met Phe Arg
 385 390 395 400

Cys Lys Lys Asn Met Glu Gly Lys Val Val Gln Pro Glu Asn Leu Glu
 405 410 415

Tyr Thr Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly

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420	425	430
Asn Asp Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser		
435	440	445
Ser Ile Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu		
450	455	460
Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln		
465	470	475
Met Glu Asn Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu		
485	490	495
Pro Leu Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile		
500	505	510
Gln Lys Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln		
515	520	525
Asp Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu		
530	535	540
Thr Gly Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr		
545	550	555
Gly His Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly		
565	570	575
Met Ser Tyr Ser Met Cys Thr Gly Lys Phe Lys Val Val Lys Glu Ile		
580	585	590
Ala Glu Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly		
595	600	605
Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys		
610	615	620
Arg His Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu		
625	630	635
Lys Asp Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser		
645	650	655
Tyr Ile Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe		
660	665	670
Lys Lys Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly		
675	680	685
Ala Lys Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser		
690	695	700
Leu Gly Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe		
705	710	715
Gly Ala Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys		
725	730	735
Ile Leu Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser		
740	745	750
Thr Ser Leu Ser Val Thr Leu Val Leu Val Gly Ile Val Thr Leu Tyr		
755	760	765
Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys		
770	775	780
Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val		
785	790	795
His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys		
805	810	815
Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile		
820	825	830
Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro		
835	840	845

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Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
850 855 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865 870 875 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
885 890 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
900 905 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
915 920 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
930 935 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945 950 955 960

Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
965 970 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
980 985 990

Ile Glu Val Lys Asn Cys His Trp Pro Lys Ser His Thr Leu Trp Ser
995 1000 1005

Asn Gly Val Leu Glu Ser Glu Met Ile Ile Pro Lys Asn Leu Ala
1010 1015 1020

Gly Pro Val Ser Gln His Asn Tyr Arg Pro Gly Tyr His Thr Gln
1025 1030 1035

Ile Thr Gly Pro Trp His Leu Gly Lys Leu Glu Met Asp Phe Asp
1040 1045 1050

Phe Cys Asp Gly Thr Thr Val Val Val Thr Glu Asp Cys Gly Asn
1055 1060 1065

Arg Gly Pro Ser Leu Arg Thr Thr Ala Ser Gly Lys Leu Ile
1070 1075 1080

Thr Glu Trp Cys Cys Arg Ser Cys Thr Leu Pro Pro Leu Arg Tyr
1085 1090 1095

Arg Gly Glu Asp Gly Cys Trp Tyr Gly Met Glu Ile Arg Pro Leu
1100 1105 1110

Lys Glu Lys Glu Glu Asn Leu Val Asn Ser Leu Val Thr Ala Gly
1115 1120 1125

His Gly Gln Val Asp Asn Phe Ser Leu Gly Val Leu Gly Met Ala
1130 1135 1140

Leu Phe Leu Glu Glu Met Leu Arg Thr Arg Val Gly Thr Lys His
1145 1150 1155

Ala Ile Leu Leu Val Ala Val Ser Phe Val Thr Leu Ile Thr Gly
1160 1165 1170

Asn Met Ser Phe Arg Asp Leu Gly Arg Val Met Val Met Val Gly
1175 1180 1185

Ala Thr Met Thr Asp Asp Ile Gly Met Gly Val Thr Tyr Leu Ala
1190 1195 1200

Leu Leu Ala Ala Phe Lys Val Arg Pro Thr Phe Ala Ala Gly Leu
1205 1210 1215

Leu Leu Arg Lys Leu Thr Ser Lys Glu Leu Met Met Thr Thr Ile
1220 1225 1230

Gly Ile Val Leu Leu Ser Gln Ser Thr Ile Pro Glu Thr Ile Leu
1235 1240 1245

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Glu Leu Thr Asp Ala Leu Ala Leu Gly Met Met Val Leu Lys Met
 1250 1255 1260
 Val Arg Asn Met Glu Lys Tyr Gln Leu Ala Val Thr Ile Met Ala
 1265 1270 1275
 Ile Leu Cys Val Pro Asn Ala Val Ile Leu Gln Asn Ala Trp Lys
 1280 1285 1290
 Val Ser Cys Thr Ile Leu Ala Val Val Ser Val Ser Pro Leu Phe
 1295 1300 1305
 Leu Thr Ser Ser Gln Gln Lys Thr Asp Trp Ile Pro Leu Ala Leu
 1310 1315 1320
 Thr Ile Lys Gly Leu Asn Pro Thr Ala Ile Phe Leu Thr Thr Leu
 1325 1330 1335
 Ser Arg Thr Ser Lys Lys Arg Ser Trp Pro Leu Asn Glu Ala Ile
 1340 1345 1350
 Met Ala Val Gly Met Val Ser Ile Leu Ala Ser Ser Leu Leu Lys
 1355 1360 1365
 Asn Asp Ile Pro Met Thr Gly Pro Leu Val Ala Gly Gly Leu Leu
 1370 1375 1380
 Thr Val Cys Tyr Val Leu Thr Gly Arg Ser Ala Asp Leu Glu Leu
 1385 1390 1395
 Glu Arg Ala Ala Asp Val Lys Trp Glu Asp Gln Ala Glu Ile Ser
 1400 1405 1410
 Gly Ser Ser Pro Ile Leu Ser Ile Thr Ile Ser Glu Asp Gly Ser
 1415 1420 1425
 Met Ser Ile Lys Asn Glu Glu Glu Glu Gln Thr Leu Thr Ile Leu
 1430 1435 1440
 Ile Arg Thr Gly Leu Leu Val Ile Ser Gly Leu Phe Pro Val Ser
 1445 1450 1455
 Ile Pro Ile Thr Ala Ala Ala Trp Tyr Leu Trp Glu Val Lys Lys
 1460 1465 1470
 Gln Arg Ala Gly Val Leu Trp Asp Val Pro Ser Pro Pro Pro Met
 1475 1480 1485
 Gly Lys Ala Glu Leu Glu Asp Gly Ala Tyr Arg Ile Lys Gln Lys
 1490 1495 1500
 Gly Ile Leu Gly Tyr Ser Gln Ile Gly Ala Gly Val Tyr Lys Glu
 1505 1510 1515
 Gly Thr Phe His Thr Met Trp His Val Thr Arg Gly Ala Val Leu
 1520 1525 1530
 Met His Lys Gly Lys Arg Ile Glu Pro Ser Trp Ala Asp Val Lys
 1535 1540 1545
 Lys Asp Leu Ile Ser Tyr Gly Gly Gly Trp Lys Leu Glu Gly Glu
 1550 1555 1560
 Trp Lys Glu Gly Glu Val Gln Val Leu Ala Leu Glu Pro Gly
 1565 1570 1575
 Lys Asn Pro Arg Ala Val Gln Thr Lys Pro Gly Leu Phe Lys Thr
 1580 1585 1590
 Asn Ala Gly Thr Ile Gly Ala Val Ser Leu Asp Phe Ser Pro Gly
 1595 1600 1605
 Thr Ser Gly Ser Pro Ile Ile Asp Lys Lys Gly Lys Val Val Gly
 1610 1615 1620
 Leu Tyr Gly Asn Gly Val Val Thr Arg Ser Gly Ala Tyr Val Ser
 1625 1630 1635
 Ala Ile Ala Gln Thr Glu Lys Ser Ile Glu Asp Asn Pro Glu Ile

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1640	1645	1650
Glu Asp Asp Ile Phe Arg Lys Arg Arg Leu Thr Ile Met Asp Leu		
1655	1660	1665
His Pro Gly Ala Gly Lys Thr Lys Arg Tyr Leu Pro Ala Ile Val		
1670	1675	1680
Arg Glu Ala Ile Lys Arg Gly Leu Arg Thr Leu Ile Leu Ala Pro		
1685	1690	1695
Thr Arg Val Val Ala Ala Glu Met Glu Glu Ala Leu Arg Gly Leu		
1700	1705	1710
Pro Ile Arg Tyr Gln Thr Pro Ala Ile Arg Ala Val His Thr Gly		
1715	1720	1725
Arg Glu Ile Val Asp Leu Met Cys His Ala Thr Phe Thr Met Arg		
1730	1735	1740
Leu Leu Ser Pro Val Arg Val Pro Asn Tyr Asn Leu Ile Ile Met		
1745	1750	1755
Asp Glu Ala His Phe Thr Asp Pro Ala Ser Ile Ala Ala Arg Gly		
1760	1765	1770
Tyr Ile Ser Thr Arg Val Glu Met Gly Glu Ala Ala Gly Ile Phe		
1775	1780	1785
Met Thr Ala Thr Pro Pro Gly Ser Arg Asp Pro Phe Pro Gln Ser		
1790	1795	1800
Asn Ala Pro Ile Ile Asp Glu Glu Arg Glu Ile Pro Glu Arg Ser		
1805	1810	1815
Trp Asn Ser Gly His Glu Trp Val Thr Asp Phe Lys Gly Lys Thr		
1820	1825	1830
Val Trp Phe Val Pro Ser Ile Lys Ala Gly Asn Asp Ile Ala Ala		
1835	1840	1845
Cys Leu Arg Lys Asn Gly Lys Lys Val Ile Gln Leu Ser Arg Lys		
1850	1855	1860
Thr Phe Asp Ser Glu Tyr Val Lys Thr Arg Thr Asn Asp Trp Asp		
1865	1870	1875
Phe Val Val Thr Thr Asp Ile Ser Glu Met Gly Ala Asn Phe Lys		
1880	1885	1890
Ala Glu Arg Val Ile Asp Pro Arg Arg Cys Met Lys Pro Val Ile		
1895	1900	1905
Leu Thr Asp Gly Glu Glu Arg Val Ile Leu Ala Gly Pro Met Pro		
1910	1915	1920
Val Thr His Ser Ser Ala Ala Gln Arg Arg Gly Arg Ile Gly Arg		
1925	1930	1935
Asn Pro Lys Asn Glu Asn Asp Gln Tyr Ile Tyr Met Gly Glu Pro		
1940	1945	1950
Leu Glu Asn Asp Glu Asp Cys Ala His Trp Lys Glu Ala Lys Met		
1955	1960	1965
Leu Leu Asp Asn Ile Asn Thr Pro Glu Gly Ile Ile Pro Ser Met		
1970	1975	1980
Phe Glu Pro Glu Arg Glu Lys Val Asp Ala Ile Asp Gly Glu Tyr		
1985	1990	1995
Arg Leu Arg Gly Glu Ala Arg Lys Thr Phe Val Asp Leu Met Arg		
2000	2005	2010
Arg Gly Asp Leu Pro Val Trp Leu Ala Tyr Arg Val Ala Ala Glu		
2015	2020	2025
Gly Ile Asn Tyr Ala Asp Arg Arg Trp Cys Phe Asp Gly Val Lys		
2030	2035	2040

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Asn	Asn	Gln	Ile	Leu	Glu	Glu	Asn	Val	Glu	Val	Glu	Ile	Trp	Thr
2045				2050					2055					
Lys	Glu	Gly	Glu	Arg	Lys	Lys	Leu	Lys	Pro	Arg	Trp	Leu	Asp	Ala
2060				2065					2070					
Arg	Ile	Tyr	Ser	Asp	Pro	Leu	Ala	Leu	Lys	Glu	Phe	Lys	Glu	Phe
2075				2080					2085					
Ala	Ala	Gly	Arg	Lys	Ser	Leu	Thr	Leu	Asn	Leu	Ile	Thr	Glu	Met
2090				2095					2100					
Gly	Arg	Leu	Pro	Thr	Phe	Met	Thr	Gln	Lys	Ala	Arg	Asp	Ala	Leu
2105				2110					2115					
Asp	Asn	Leu	Ala	Val	Leu	His	Thr	Ala	Glu	Ala	Gly	Gly	Arg	Ala
2120				2125					2130					
Tyr	Asn	His	Ala	Leu	Ser	Glu	Leu	Pro	Glu	Thr	Leu	Glu	Thr	Leu
2135				2140					2145					
Leu	Leu	Leu	Thr	Leu	Leu	Ala	Thr	Val	Thr	Gly	Gly	Ile	Phe	Leu
2150				2155					2160					
Phe	Leu	Met	Ser	Ala	Arg	Gly	Ile	Gly	Lys	Met	Thr	Leu	Gly	Met
2165				2170					2175					
Cys	Cys	Ile	Ile	Thr	Ala	Ser	Ile	Leu	Leu	Trp	Tyr	Ala	Gln	Ile
2180				2185					2190					
Gln	Pro	His	Trp	Ile	Ala	Ala	Ser	Ile	Ile	Leu	Glu	Phe	Phe	Leu
2195				2200					2205					
Ile	Val	Leu	Leu	Ile	Pro	Glu	Pro	Glu	Lys	Gln	Arg	Thr	Pro	Gln
2210				2215					2220					
Asp	Asn	Gln	Leu	Thr	Tyr	Val	Val	Ile	Ala	Ile	Leu	Thr	Val	Val
2225				2230					2235					
Ala	Ala	Thr	Met	Ala	Asn	Glu	Met	Gly	Phe	Leu	Glu	Lys	Thr	Lys
2240				2245					2250					
Lys	Asp	Leu	Gly	Leu	Gly	Ser	Ile	Ala	Thr	Gln	Gln	Pro	Glu	Ser
2255				2260					2265					
Asn	Ile	Leu	Asp	Ile	Asp	Leu	Arg	Pro	Ala	Ser	Ala	Trp	Thr	Leu
2270				2275					2280					
Tyr	Ala	Val	Ala	Thr	Thr	Phe	Val	Thr	Pro	Met	Leu	Arg	His	Ser
2285				2290					2295					
Ile	Glu	Asn	Ser	Ser	Val	Asn	Val	Ser	Leu	Thr	Ala	Ile	Ala	Asn
2300				2305					2310					
Gln	Ala	Thr	Val	Leu	Met	Gly	Leu	Gly	Lys	Gly	Trp	Pro	Leu	Ser
2315				2320					2325					
Lys	Met	Asp	Ile	Gly	Val	Pro	Leu	Leu	Ala	Ile	Gly	Cys	Tyr	Ser
2330				2335					2340					
Gln	Val	Asn	Pro	Ile	Thr	Leu	Thr	Ala	Ala	Leu	Phe	Leu	Leu	Val
2345				2350					2355					
Ala	His	Tyr	Ala	Ile	Ile	Gly	Pro	Gly	Leu	Gln	Ala	Lys	Ala	Thr
2360				2365					2370					
Arg	Glu	Ala	Gln	Lys	Arg	Ala	Ala	Ala	Gly	Ile	Met	Lys	Asn	Pro
2375				2380					2385					
Thr	Val	Asp	Gly	Ile	Thr	Val	Ile	Asp	Leu	Asp	Pro	Ile	Pro	Tyr
2390				2395					2400					
Asp	Pro	Lys	Phe	Glu	Lys	Gln	Leu	Gly	Gln	Val	Met	Leu	Leu	Val
2405				2410					2415					
Leu	Cys	Val	Thr	Gln	Val	Leu	Met	Met	Arg	Thr	Trp	Ala	Leu	
2420				2425					2430					

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Cys Glu Ala Leu Thr Leu Ala Thr Gly Pro Ile Ser Thr Leu Trp
2435 2440 2445

Glu Gly Asn Pro Gly Arg Phe Trp Asn Thr Thr Ile Ala Val Ser
2450 2455 2460

Met Ala Asn Ile Phe Arg Gly Ser Tyr Leu Ala Gly Ala Gly Leu
2465 2470 2475

Leu Phe Ser Ile Met Lys Asn Thr Thr Asn Thr Arg Arg Gly Thr
2480 2485 2490

Gly Asn Ile Gly Glu Thr Leu Gly Glu Lys Trp Lys Ser Arg Leu
2495 2500 2505

Asn Ala Leu Gly Lys Ser Glu Phe Gln Ile Tyr Lys Lys Ser Gly
2510 2515 2520

Ile Gln Glu Val Asp Arg Thr Leu Ala Lys Glu Gly Ile Lys Arg
2525 2530 2535

Gly Glu Thr Asp His His Ala Val Ser Arg Gly Ser Ala Lys Leu
2540 2545 2550

Arg Trp Phe Val Glu Arg Asn Met Val Thr Pro Glu Gly Lys Val
2555 2560 2565

Val Asp Leu Gly Cys Gly Arg Gly Gly Trp Ser Tyr Tyr Cys Gly
2570 2575 2580

Gly Leu Lys Asn Val Arg Glu Val Lys Gly Leu Thr Lys Gly Gly
2585 2590 2595

Pro Gly His Glu Glu Pro Ile Pro Met Ser Thr Tyr Gly Trp Asn
2600 2605 2610

Leu Val Arg Leu Gln Ser Gly Val Asp Val Phe Phe Ile Pro Pro
2615 2620 2625

Glu Lys Cys Asp Thr Leu Leu Cys Asp Ile Gly Glu Ser Ser Pro
2630 2635 2640

Asn Pro Thr Val Glu Ala Gly Arg Thr Leu Arg Val Leu Asn Leu
2645 2650 2655

Val Glu Asn Trp Leu Asn Asn Asn Thr Gln Phe Cys Ile Lys Val
2660 2665 2670

Leu Asn Pro Tyr Met Pro Ser Val Ile Glu Lys Met Glu Ala Leu
2675 2680 2685

Gln Arg Lys Tyr Gly Gly Ala Leu Val Arg Asn Pro Leu Ser Arg
2690 2695 2700

Asn Ser Thr His Glu Met Tyr Trp Val Ser Asn Ala Ser Gly Asn
2705 2710 2715

Ile Val Ser Ser Val Asn Met Ile Ser Arg Met Leu Ile Asn Arg
2720 2725 2730

Phe Thr Met Arg Tyr Lys Lys Ala Thr Tyr Glu Pro Asp Val Asp
2735 2740 2745

Leu Gly Ser Gly Thr Arg Asn Ile Gly Ile Glu Ser Glu Ile Pro
2750 2755 2760

Asn Leu Asp Ile Ile Gly Lys Arg Ile Glu Lys Ile Lys Gln Glu
2765 2770 2775

His Glu Thr Ser Trp His Tyr Asp Gln Asp His Pro Tyr Lys Thr
2780 2785 2790

Trp Ala Tyr His Gly Ser Tyr Glu Thr Lys Gln Thr Gly Ser Ala
2795 2800 2805

Ser Ser Met Val Asn Gly Val Val Arg Leu Leu Thr Lys Pro Trp
2810 2815 2820

Asp Val Val Pro Met Val Thr Gln Met Ala Met Thr Asp Thr Thr

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2825	2830	2835
Pro Phe Gly Gln Gln Arg Val Phe Lys Glu Lys Val Asp Thr Arg		
2840	2845	2850
Thr Gln Glu Pro Lys Glu Gly Thr Lys Lys Leu Met Lys Ile Thr		
2855	2860	2865
Ala Glu Trp Leu Trp Lys Glu Leu Gly Lys Lys Thr Pro Arg		
2870	2875	2880
Met Cys Thr Arg Glu Glu Phe Thr Arg Lys Val Arg Ser Asn Ala		
2885	2890	2895
Ala Leu Gly Ala Val Phe Thr Asp Glu Asn Lys Trp Lys Ser Ala		
2900	2905	2910
Arg Glu Ala Val Glu Asp Ser Arg Phe Trp Glu Leu Val Asp Lys		
2915	2920	2925
Glu Arg Asn Leu His Leu Glu Gly Lys Cys Glu Thr Cys Val Tyr		
2930	2935	2940
Asn Met Met Gly Lys Arg Glu Lys Lys Leu Gly Glu Phe Gly Lys		
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Ala Lys Gly Ser Arg Ala Ile Trp Tyr Met Trp Leu Gly Ala Arg		
2960	2965	2970
Phe Leu Glu Phe Glu Ala Leu Gly Phe Leu Asn Glu Asp His Trp		
2975	2980	2985
Phe Ser Arg Glu Asn Ser Leu Ser Gly Val Glu Gly Glu Gly Leu		
2990	2995	3000
His Lys Leu Gly Tyr Ile Leu Arg Asp Val Ser Lys Lys Glu Gly		
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Gly Ala Met Tyr Ala Asp Asp Thr Ala Gly Trp Asp Thr Arg Ile		
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Thr Leu Glu Asp Leu Lys Asn Glu Glu Met Val Thr Asn His Met		
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Glu Gly Glu His Lys Lys Leu Ala Glu Ala Ile Phe Lys Leu Thr		
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Tyr Gln Asn Lys Val Val Arg Val Gln Arg Pro Thr Pro Arg Gly		
3065	3070	3075
Thr Val Met Asp Ile Ile Ser Arg Arg Asp Gln Arg Gly Ser Gly		
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Gln Val Gly Thr Tyr Gly Leu Asn Thr Phe Thr Asn Met Glu Ala		
3095	3100	3105
Gln Leu Ile Arg Gln Met Glu Gly Glu Gly Val Phe Lys Ser Ile		
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Gln His Leu Thr Ile Thr Glu Glu Ile Ala Val Gln Asn Trp Leu		
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Ala Arg Val Gly Arg Glu Arg Leu Ser Arg Met Ala Ile Ser Gly		
3140	3145	3150
Asp Asp Cys Val Val Lys Pro Leu Asp Asp Arg Phe Ala Ser Ala		
3155	3160	3165
Leu Thr Ala Leu Asn Asp Met Gly Lys Ile Arg Lys Asp Ile Gln		
3170	3175	3180
Gln Trp Glu Pro Ser Arg Gly Trp Asn Asp Trp Thr Gln Val Pro		
3185	3190	3195
Phe Cys Ser His His Phe His Glu Leu Ile Met Lys Asp Gly Arg		
3200	3205	3210
Val Leu Val Val Pro Cys Arg Asn Gln Asp Glu Leu Ile Gly Arg		
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 His Arg Arg Asp Leu Arg Leu Ala Ala Asn Ala Ile Cys Ser Ala
 3260 3265 3270
 Val Pro Ser His Trp Val Pro Thr Ser Arg Thr Trp Ser Ile
 3275 3280 3285
 His Ala Lys His Glu Trp Met Thr Thr Glu Asp Met Leu Thr Val
 3290 3295 3300
 Trp Asn Arg Val Trp Ile Gln Glu Asn Pro Trp Met Glu Asp Lys
 3305 3310 3315
 Thr Pro Val Glu Ser Trp Glu Glu Ile Pro Tyr Leu Gly Lys Arg
 3320 3325 3330
 Glu Asp Gln Trp Cys Gly Ser Leu Ile Gly Leu Thr Ser Arg Ala
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 Thr Trp Ala Lys Asn Ile Gln Ala Ala Ile Asn Gln Val Arg Ser
 3350 3355 3360
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 3380 3385 3390

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 <211> LENGTH: 10717
 <212> TYPE: DNA
 <213> ORGANISM: chimeric dengue serotype 2/3 (MVS)

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<210> SEQ_ID NO 6
 <211> LENGTH: 3389
 <212> TYPE: PRT
 <213> ORGANISM: chimeric dengue serotype 2/3 (MVS)

<400> SEQUENCE: 6

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Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg		
20	25	30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met		
35	40	45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly		
50	55	60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Ser Lys Ala Ile Asn Val			
65	70	75	80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn		
85	90	95

Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val		
100	105	110

Met Ala Phe His Leu Thr Thr Arg Asp Gly Glu Pro Arg Met Ile Val		
115	120	125

Gly Lys Asn Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ala Ser Gly		
130	135	140

Ile Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Asp			
145	150	155	160

Asp Thr Val Thr Tyr Lys Cys Pro His Ile Thr Glu Val Glu Pro Glu		
165	170	175

Asp Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Thr Tyr Gly		
180	185	190

Thr Cys Asn Gln Ala Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala		
195	200	205

Leu Ala Pro His Val Gly Met Gly Leu Asp Thr Arg Thr Gln Thr Trp		
210	215	220

Met Ser Ala Glu Gly Ala Trp Arg Gln Val Glu Lys Val Glu Thr Trp			
225	230	235	240

Ala Leu Arg His Pro Gly Phe Thr Ile Leu Ala Leu Phe Leu Ala His		
245	250	255

Tyr Ile Gly Thr Ser Leu Thr Gln Lys Val Val Ile Phe Ile Leu Leu		
260	265	270

Met Leu Val Thr Pro Ser Met Thr Met Arg Cys Val Gly Val Gly Asn		
275	280	285

Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val		
290	295	300

Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr			
305	310	315	320

Leu Asp Ile Glu Leu Gln Lys Thr Glu Ala Thr Gln Leu Ala Thr Leu		
325	330	335

Arg Lys Leu Cys Ile Glu Gly Lys Ile Thr Asn Ile Thr Thr Asp Ser		
340	345	350

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 385 390 395 400
 Cys Leu Glu Ser Ile Glu Gly Lys Val Val Gln His Glu Asn Leu Lys
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 420 425 430
 Asn Glu Thr Gln Gly Val Thr Ala Glu Ile Thr Pro Gln Ala Ser Thr
 435 440 445
 Ala Glu Ala Ile Leu Pro Glu Tyr Gly Thr Leu Gly Leu Glu Cys Ser
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 Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Ile Ser Leu Thr Met Lys
 465 470 475 480
 Asn Lys Ala Trp Met Val His Arg Gln Trp Phe Phe Asp Leu Pro Leu
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 Pro Trp Thr Ser Gly Ala Ser Ala Glu Thr Pro Thr Trp Asn Arg Lys
 500 505 510
 Glu Leu Leu Val Thr Phe Lys Asn Ala His Ala Lys Lys Gln Glu Val
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 Ala Thr Glu Ile Gln Thr Ser Gly Gly Thr Ser Ile Phe Ala Gly His
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 Tyr Ala Met Cys Leu Ser Ser Phe Val Leu Lys Lys Glu Val Ser Glu
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 Thr Gln His Gly Thr Ile Leu Ile Lys Val Glu Tyr Lys Gly Glu Asp
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 625 630 635 640
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 645 650 655
 Val Ile Gly Ile Gly Asp Lys Ala Leu Lys Ile Asn Trp Tyr Lys Lys
 660 665 670
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 675 680 685
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 Ala Tyr Thr Ala Leu Phe Gly Gly Val Ser Trp Met Met Lys Ile Gly
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 Ile Gly Val Leu Leu Thr Trp Ile Gly Leu Asn Ser Lys Asn Thr Ser
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Val	Thr	Arg	Leu	Glu	Asn	Leu	Met	Trp	Lys	Gln	Ile	Thr	Pro	Glu	Leu
				835			840					845			
Asn	His	Ile	Leu	Ser	Glu	Asn	Glu	Val	Lys	Leu	Thr	Ile	Met	Thr	Gly
				850			855					860			
Asp	Ile	Lys	Gly	Ile	Met	Gln	Ala	Gly	Lys	Arg	Ser	Leu	Arg	Pro	Gln
				865			870			875			880		
Pro	Thr	Glu	Leu	Lys	Tyr	Ser	Trp	Lys	Thr	Trp	Gly	Lys	Ala	Lys	Met
				885			890					895			
Leu	Ser	Thr	Glu	Ser	His	Asn	Gln	Thr	Phe	Leu	Ile	Asp	Gly	Pro	Glu
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Thr	Ala	Glu	Cys	Pro	Asn	Thr	Asn	Arg	Ala	Trp	Asn	Ser	Leu	Glu	Val
				915			920					925			
Glu	Asp	Tyr	Gly	Phe	Gly	Val	Phe	Thr	Thr	Asn	Ile	Trp	Leu	Lys	Leu
				930			935					940			
Lys	Glu	Lys	Gln	Asp	Val	Phe	Cys	Asp	Ser	Lys	Leu	Met	Ser	Ala	Ala
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Ile	Lys	Asp	Asn	Arg	Ala	Val	His	Ala	Asp	Met	Gly	Tyr	Trp	Ile	Glu
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Ser	Ala	Leu	Asn	Asp	Thr	Trp	Lys	Ile	Glu	Lys	Ala	Ser	Phe	Ile	Glu
				980			985					990			
Val	Lys	Asn	Cys	His	Trp	Pro	Lys	Ser	His	Thr	Leu	Trp	Ser	Asn	Gly
				995			1000					1005			
Val	Leu	Glu	Ser	Glu	Met	Ile	Ile	Pro	Lys	Asn	Leu	Ala	Gly	Pro	
				1010			1015					1020			
Val	Ser	Gln	His	Asn	Tyr	Arg	Pro	Gly	Tyr	His	Thr	Gln	Ile	Thr	
				1025			1030					1035			
Gly	Pro	Trp	His	Leu	Gly	Lys	Leu	Glu	Met	Asp	Phe	Asp	Phe	Cys	
				1040			1045					1050			
Asp	Gly	Thr	Thr	Val	Val	Thr	Glu	Asp	Cys	Gly	Asn	Arg	Gly		
				1055			1060					1065			
Pro	Ser	Leu	Arg	Thr	Thr	Ala	Ser	Gly	Lys	Leu	Ile	Thr	Glu		
				1070			1075					1080			
Trp	Cys	Cys	Arg	Ser	Cys	Thr	Leu	Pro	Pro	Leu	Arg	Tyr	Arg	Gly	
				1085			1090					1095			
Glu	Asp	Gly	Cys	Trp	Tyr	Gly	Met	Glu	Ile	Arg	Pro	Leu	Lys	Glu	
				1100			1105					1110			
Lys	Glu	Glu	Asn	Leu	Val	Asn	Ser	Leu	Val	Thr	Ala	Gly	His	Gly	
				1115			1120					1125			
Gln	Val	Asp	Asn	Phe	Ser	Leu	Gly	Val	Leu	Gly	Met	Ala	Leu	Phe	
				1130			1135					1140			
Leu	Glu	Glu	Met	Leu	Arg	Thr	Arg	Val	Gly	Thr	Lys	His	Ala	Ile	
				1145			1150					1155			
Leu	Leu	Val	Ala	Val	Ser	Phe	Val	Thr	Leu	Ile	Thr	Gly	Asn	Met	
				1160			1165					1170			
Ser	Phe	Arg	Asp	Leu	Gly	Arg	Val	Met	Val	Met	Val	Gly	Ala	Thr	
				1175			1180					1185			

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Met Thr Asp Asp Ile Gly Met Gly Val Thr Tyr Leu Ala Leu Leu
 1190 1195 1200
 Ala Ala Phe Lys Val Arg Pro Thr Phe Ala Ala Gly Leu Leu Leu
 1205 1210 1215
 Arg Lys Leu Thr Ser Lys Glu Leu Met Met Thr Thr Ile Gly Ile
 1220 1225 1230
 Val Leu Leu Ser Gln Ser Thr Ile Pro Glu Thr Ile Leu Glu Leu
 1235 1240 1245
 Thr Asp Ala Leu Ala Leu Gly Met Met Val Leu Lys Met Val Arg
 1250 1255 1260
 Asn Met Glu Lys Tyr Gln Leu Ala Val Thr Ile Met Ala Ile Leu
 1265 1270 1275
 Cys Val Pro Asn Ala Val Ile Leu Gln Asn Ala Trp Lys Val Ser
 1280 1285 1290
 Cys Thr Ile Leu Ala Val Val Ser Val Ser Pro Leu Phe Leu Thr
 1295 1300 1305
 Ser Ser Gln Gln Lys Thr Asp Trp Ile Pro Leu Ala Leu Thr Ile
 1310 1315 1320
 Lys Gly Leu Asn Pro Thr Ala Ile Phe Leu Thr Thr Leu Ser Arg
 1325 1330 1335
 Thr Ser Lys Lys Arg Ser Trp Pro Leu Asn Glu Ala Ile Met Ala
 1340 1345 1350
 Val Gly Met Val Ser Ile Leu Ala Ser Ser Leu Leu Lys Asn Asp
 1355 1360 1365
 Ile Pro Met Thr Gly Pro Leu Val Ala Gly Leu Leu Thr Val
 1370 1375 1380
 Cys Tyr Val Leu Thr Gly Arg Ser Ala Asp Leu Glu Leu Glu Arg
 1385 1390 1395
 Ala Ala Asp Val Lys Trp Glu Asp Gln Ala Glu Ile Ser Gly Ser
 1400 1405 1410
 Ser Pro Ile Leu Ser Ile Thr Ile Ser Glu Asp Gly Ser Met Ser
 1415 1420 1425
 Ile Lys Asn Glu Glu Glu Gln Thr Leu Thr Ile Leu Ile Arg
 1430 1435 1440
 Thr Gly Leu Leu Val Ile Ser Gly Leu Phe Pro Val Ser Ile Pro
 1445 1450 1455
 Ile Thr Ala Ala Ala Trp Tyr Leu Trp Glu Val Lys Lys Gln Arg
 1460 1465 1470
 Ala Gly Val Leu Trp Asp Val Pro Ser Pro Pro Pro Met Gly Lys
 1475 1480 1485
 Ala Glu Leu Glu Asp Gly Ala Tyr Arg Ile Lys Gln Lys Gly Ile
 1490 1495 1500
 Leu Gly Tyr Ser Gln Ile Gly Ala Gly Val Tyr Lys Glu Gly Thr
 1505 1510 1515
 Phe His Thr Met Trp His Val Thr Arg Gly Ala Val Leu Met His
 1520 1525 1530
 Lys Gly Lys Arg Ile Glu Pro Ser Trp Ala Asp Val Lys Lys Asp
 1535 1540 1545
 Leu Ile Ser Tyr Gly Gly Trp Lys Leu Glu Gly Glu Trp Lys
 1550 1555 1560
 Glu Gly Glu Glu Val Gln Val Leu Ala Leu Glu Pro Gly Lys Asn
 1565 1570 1575

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Pro Arg Ala Val Gln Thr Lys Pro Gly Leu Phe Lys Thr Asn Ala
 1580 1585 1590
 Gly Thr Ile Gly Ala Val Ser Leu Asp Phe Ser Pro Gly Thr Ser
 1595 1600 1605
 Gly Ser Pro Ile Ile Asp Lys Lys Gly Lys Val Val Gly Leu Tyr
 1610 1615 1620
 Gly Asn Gly Val Val Thr Arg Ser Gly Ala Tyr Val Ser Ala Ile
 1625 1630 1635
 Ala Gln Thr Glu Lys Ser Ile Glu Asp Asn Pro Glu Ile Glu Asp
 1640 1645 1650
 Asp Ile Phe Arg Lys Arg Arg Leu Thr Ile Met Asp Leu His Pro
 1655 1660 1665
 Gly Ala Gly Lys Thr Lys Arg Tyr Leu Pro Ala Ile Val Arg Glu
 1670 1675 1680
 Ala Ile Lys Arg Gly Leu Arg Thr Leu Ile Leu Ala Pro Thr Arg
 1685 1690 1695
 Val Val Ala Ala Glu Met Glu Glu Ala Leu Arg Gly Leu Pro Ile
 1700 1705 1710
 Arg Tyr Gln Thr Pro Ala Ile Arg Ala Val His Thr Gly Arg Glu
 1715 1720 1725
 Ile Val Asp Leu Met Cys His Ala Thr Phe Thr Met Arg Leu Leu
 1730 1735 1740
 Ser Pro Val Arg Val Pro Asn Tyr Asn Leu Ile Ile Met Asp Glu
 1745 1750 1755
 Ala His Phe Thr Asp Pro Ala Ser Ile Ala Ala Arg Gly Tyr Ile
 1760 1765 1770
 Ser Thr Arg Val Glu Met Gly Glu Ala Ala Gly Ile Phe Met Thr
 1775 1780 1785
 Ala Thr Pro Pro Gly Ser Arg Asp Pro Phe Pro Gln Ser Asn Ala
 1790 1795 1800
 Pro Ile Ile Asp Glu Glu Arg Glu Ile Pro Glu Arg Ser Trp Asn
 1805 1810 1815
 Ser Gly His Glu Trp Val Thr Asp Phe Lys Gly Lys Thr Val Trp
 1820 1825 1830
 Phe Val Pro Ser Ile Lys Ala Gly Asn Asp Ile Ala Ala Cys Leu
 1835 1840 1845
 Arg Lys Asn Gly Lys Lys Val Ile Gln Leu Ser Arg Lys Thr Phe
 1850 1855 1860
 Asp Ser Glu Tyr Val Lys Thr Arg Thr Asn Asp Trp Asp Phe Val
 1865 1870 1875
 Val Thr Thr Asp Ile Ser Glu Met Gly Ala Asn Phe Lys Ala Glu
 1880 1885 1890
 Arg Val Ile Asp Pro Arg Arg Cys Met Lys Pro Val Ile Leu Thr
 1895 1900 1905
 Asp Gly Glu Glu Arg Val Ile Leu Ala Gly Pro Met Pro Val Thr
 1910 1915 1920
 His Ser Ser Ala Ala Gln Arg Arg Gly Arg Ile Gly Arg Asn Pro
 1925 1930 1935
 Lys Asn Glu Asn Asp Gln Tyr Ile Tyr Met Gly Glu Pro Leu Glu
 1940 1945 1950
 Asn Asp Glu Asp Cys Ala His Trp Lys Glu Ala Lys Met Leu Leu
 1955 1960 1965
 Asp Asn Ile Asn Thr Pro Glu Gly Ile Ile Pro Ser Met Phe Glu

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1970	1975	1980
Pro Glu Arg Glu Lys Val Asp Ala Ile Asp Gly Glu Tyr Arg Leu		
1985 1990 1995		
Arg Gly Glu Ala Arg Lys Thr Phe Val Asp Leu Met Arg Arg Gly		
2000 2005 2010		
Asp Leu Pro Val Trp Leu Ala Tyr Arg Val Ala Ala Glu Gly Ile		
2015 2020 2025		
Asn Tyr Ala Asp Arg Arg Trp Cys Phe Asp Gly Val Lys Asn Asn		
2030 2035 2040		
Gln Ile Leu Glu Glu Asn Val Glu Val Glu Ile Trp Thr Lys Glu		
2045 2050 2055		
Gly Glu Arg Lys Lys Leu Lys Pro Arg Trp Leu Asp Ala Arg Ile		
2060 2065 2070		
Tyr Ser Asp Pro Leu Ala Leu Lys Glu Phe Lys Glu Phe Ala Ala		
2075 2080 2085		
Gly Arg Lys Ser Leu Thr Leu Asn Leu Ile Thr Glu Met Gly Arg		
2090 2095 2100		
Leu Pro Thr Phe Met Thr Gln Lys Ala Arg Asp Ala Leu Asp Asn		
2105 2110 2115		
Leu Ala Val Leu His Thr Ala Glu Ala Gly Gly Arg Ala Tyr Asn		
2120 2125 2130		
His Ala Leu Ser Glu Leu Pro Glu Thr Leu Glu Thr Leu Leu Leu		
2135 2140 2145		
Leu Thr Leu Leu Ala Thr Val Thr Gly Gly Ile Phe Leu Phe Leu		
2150 2155 2160		
Met Ser Ala Arg Gly Ile Gly Lys Met Thr Leu Gly Met Cys Cys		
2165 2170 2175		
Ile Ile Thr Ala Ser Ile Leu Leu Trp Tyr Ala Gln Ile Gln Pro		
2180 2185 2190		
His Trp Ile Ala Ala Ser Ile Ile Leu Glu Phe Phe Leu Ile Val		
2195 2200 2205		
Leu Leu Ile Pro Glu Pro Glu Lys Gln Arg Thr Pro Gln Asp Asn		
2210 2215 2220		
Gln Leu Thr Tyr Val Val Ile Ala Ile Leu Thr Val Val Ala Ala		
2225 2230 2235		
Thr Met Ala Asn Glu Met Gly Phe Leu Glu Lys Thr Lys Lys Asp		
2240 2245 2250		
Leu Gly Leu Gly Ser Ile Ala Thr Gln Gln Pro Glu Ser Asn Ile		
2255 2260 2265		
Leu Asp Ile Asp Leu Arg Pro Ala Ser Ala Trp Thr Leu Tyr Ala		
2270 2275 2280		
Val Ala Thr Thr Phe Val Thr Pro Met Leu Arg His Ser Ile Glu		
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Asn Ser Ser Val Asn Val Ser Leu Thr Ala Ile Ala Asn Gln Ala		
2300 2305 2310		
Thr Val Leu Met Gly Leu Gly Lys Gly Trp Pro Leu Ser Lys Met		
2315 2320 2325		
Asp Ile Gly Val Pro Leu Leu Ala Ile Gly Cys Tyr Ser Gln Val		
2330 2335 2340		
Asn Pro Ile Thr Leu Thr Ala Ala Leu Phe Leu Leu Val Ala His		
2345 2350 2355		
Tyr Ala Ile Ile Gly Pro Gly Leu Gln Ala Lys Ala Thr Arg Glu		
2360 2365 2370		

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Ala Gln Lys Arg Ala Ala Ala Gly Ile Met Lys Asn Pro Thr Val
 2375 2380 2385
 Asp Gly Ile Thr Val Ile Asp Leu Asp Pro Ile Pro Tyr Asp Pro
 2390 2395 2400
 Lys Phe Glu Lys Gln Leu Gly Gln Val Met Leu Leu Val Leu Cys
 2405 2410 2415
 Val Thr Gln Val Leu Met Met Arg Thr Thr Trp Ala Leu Cys Glu
 2420 2425 2430
 Ala Leu Thr Leu Ala Thr Gly Pro Ile Ser Thr Leu Trp Glu Gly
 2435 2440 2445
 Asn Pro Gly Arg Phe Trp Asn Thr Thr Ile Ala Val Ser Met Ala
 2450 2455 2460
 Asn Ile Phe Arg Gly Ser Tyr Leu Ala Gly Ala Gly Leu Leu Phe
 2465 2470 2475
 Ser Ile Met Lys Asn Thr Thr Asn Thr Arg Arg Gly Thr Gly Asn
 2480 2485 2490
 Ile Gly Glu Thr Leu Gly Glu Lys Trp Lys Ser Arg Leu Asn Ala
 2495 2500 2505
 Leu Gly Lys Ser Glu Phe Gln Ile Tyr Lys Ser Gly Ile Gln
 2510 2515 2520
 Glu Val Asp Arg Thr Leu Ala Lys Glu Gly Ile Lys Arg Gly Glu
 2525 2530 2535
 Thr Asp His His Ala Val Ser Arg Gly Ser Ala Lys Leu Arg Trp
 2540 2545 2550
 Phe Val Glu Arg Asn Met Val Thr Pro Glu Gly Lys Val Val Asp
 2555 2560 2565
 Leu Gly Cys Gly Arg Gly Gly Trp Ser Tyr Tyr Cys Gly Gly Leu
 2570 2575 2580
 Lys Asn Val Arg Glu Val Lys Gly Leu Thr Lys Gly Gly Pro Gly
 2585 2590 2595
 His Glu Glu Pro Ile Pro Met Ser Thr Tyr Gly Trp Asn Leu Val
 2600 2605 2610
 Arg Leu Gln Ser Gly Val Asp Val Phe Phe Ile Pro Pro Glu Lys
 2615 2620 2625
 Cys Asp Thr Leu Leu Cys Asp Ile Gly Glu Ser Ser Pro Asn Pro
 2630 2635 2640
 Thr Val Glu Ala Gly Arg Thr Leu Arg Val Leu Asn Leu Val Glu
 2645 2650 2655
 Asn Trp Leu Asn Asn Asn Thr Gln Phe Cys Ile Lys Val Leu Asn
 2660 2665 2670
 Pro Tyr Met Pro Ser Val Ile Glu Lys Met Glu Ala Leu Gln Arg
 2675 2680 2685
 Lys Tyr Gly Gly Ala Leu Val Arg Asn Pro Leu Ser Arg Asn Ser
 2690 2695 2700
 Thr His Glu Met Tyr Trp Val Ser Asn Ala Ser Gly Asn Ile Val
 2705 2710 2715
 Ser Ser Val Asn Met Ile Ser Arg Met Leu Ile Asn Arg Phe Thr
 2720 2725 2730
 Met Arg Tyr Lys Lys Ala Thr Tyr Glu Pro Asp Val Asp Leu Gly
 2735 2740 2745
 Ser Gly Thr Arg Asn Ile Gly Ile Glu Ser Glu Ile Pro Asn Leu
 2750 2755 2760

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Asp Ile Ile Gly Lys Arg Ile Glu Lys Ile Lys Gln Glu His Glu
 2765 2770 2775
 Thr Ser Trp His Tyr Asp Gln Asp His Pro Tyr Lys Thr Trp Ala
 2780 2785 2790
 Tyr His Gly Ser Tyr Glu Thr Lys Gln Thr Gly Ser Ala Ser Ser
 2795 2800 2805
 Met Val Asn Gly Val Val Arg Leu Leu Thr Lys Pro Trp Asp Val
 2810 2815 2820
 Val Pro Met Val Thr Gln Met Ala Met Thr Asp Thr Thr Pro Phe
 2825 2830 2835
 Gly Gln Gln Arg Val Phe Lys Glu Lys Val Asp Thr Arg Thr Gln
 2840 2845 2850
 Glu Pro Lys Glu Gly Thr Lys Lys Leu Met Lys Ile Thr Ala Glu
 2855 2860 2865
 Trp Leu Trp Lys Glu Leu Gly Lys Lys Lys Thr Pro Arg Met Cys
 2870 2875 2880
 Thr Arg Glu Glu Phe Thr Arg Lys Val Arg Ser Asn Ala Ala Leu
 2885 2890 2895
 Gly Ala Ile Phe Thr Asp Glu Asn Lys Trp Lys Ser Ala Arg Glu
 2900 2905 2910
 Ala Val Glu Asp Ser Arg Phe Trp Glu Leu Val Asp Lys Glu Arg
 2915 2920 2925
 Asn Leu His Leu Glu Gly Lys Cys Glu Thr Cys Val Tyr Asn Met
 2930 2935 2940
 Met Gly Lys Arg Glu Lys Lys Leu Gly Glu Phe Gly Lys Ala Lys
 2945 2950 2955
 Gly Ser Arg Ala Ile Trp Tyr Met Trp Leu Gly Ala Arg Phe Leu
 2960 2965 2970
 Glu Phe Glu Ala Leu Gly Phe Leu Asn Glu Asp His Trp Phe Ser
 2975 2980 2985
 Arg Glu Asn Ser Leu Ser Gly Val Glu Gly Glu Leu His Lys
 2990 2995 3000
 Leu Gly Tyr Ile Leu Arg Asp Val Ser Lys Glu Gly Gly Ala
 3005 3010 3015
 Met Tyr Ala Asp Asp Thr Ala Gly Trp Asp Thr Arg Ile Thr Leu
 3020 3025 3030
 Glu Asp Leu Lys Asn Glu Glu Met Val Thr Asn His Met Glu Gly
 3035 3040 3045
 Glu His Lys Lys Leu Ala Glu Ala Ile Phe Lys Leu Thr Tyr Gln
 3050 3055 3060
 Asn Lys Val Val Arg Val Gln Arg Pro Thr Pro Arg Gly Thr Val
 3065 3070 3075
 Met Asp Ile Ile Ser Arg Arg Asp Gln Arg Gly Ser Gly Gln Val
 3080 3085 3090
 Gly Thr Tyr Gly Leu Asn Thr Phe Thr Asn Met Glu Ala Gln Leu
 3095 3100 3105
 Ile Arg Gln Met Glu Gly Glu Gly Val Phe Lys Ser Ile Gln His
 3110 3115 3120
 Leu Thr Ile Thr Glu Glu Ile Ala Val Gln Asn Trp Leu Ala Arg
 3125 3130 3135
 Val Gly Arg Glu Arg Leu Ser Arg Met Ala Ile Ser Gly Asp Asp
 3140 3145 3150
 Cys Val Val Lys Pro Leu Asp Asp Arg Phe Ala Ser Ala Leu Thr

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3155	3160	3165
Ala Leu Asn Asp Met Gly Lys Ile Arg Lys Asp Ile Gln Gln Trp		
3170	3175	3180
Glu Pro Ser Arg Gly Trp Asn Asp Trp Thr Gln Val Pro Phe Cys		
3185	3190	3195
Ser His His Phe His Glu Leu Ile Met Lys Asp Gly Arg Val Leu		
3200	3205	3210
Val Val Pro Cys Arg Asn Gln Asp Glu Leu Ile Gly Arg Ala Arg		
3215	3220	3225
Ile Ser Gln Gly Ala Gly Trp Ser Leu Arg Glu Thr Ala Cys Leu		
3230	3235	3240
Gly Lys Ser Tyr Ala Gln Met Trp Ser Leu Met Tyr Phe His Arg		
3245	3250	3255
Arg Asp Leu Arg Leu Ala Ala Asn Ala Ile Cys Ser Ala Val Pro		
3260	3265	3270
Ser His Trp Val Pro Thr Ser Arg Thr Thr Trp Ser Ile His Ala		
3275	3280	3285
Lys His Glu Trp Met Thr Thr Glu Asp Met Leu Thr Val Trp Asn		
3290	3295	3300
Arg Val Trp Ile Gln Glu Asn Pro Trp Met Glu Asp Lys Thr Pro		
3305	3310	3315
Val Glu Ser Trp Glu Glu Ile Pro Tyr Leu Gly Lys Arg Glu Asp		
3320	3325	3330
Gln Trp Cys Gly Ser Leu Ile Gly Leu Thr Ser Arg Ala Thr Trp		
3335	3340	3345
Ala Lys Asn Ile Gln Ala Ala Ile Asn Gln Val Arg Ser Leu Ile		
3350	3355	3360
Gly Asn Glu Glu Tyr Thr Asp Tyr Met Pro Ser Met Lys Arg Phe		
3365	3370	3375
Arg Arg Glu Glu Glu Ala Gly Val Leu Trp		
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<223> OTHER INFORMATION: t or c

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 <211> LENGTH: 3391
 <212> TYPE: PRT
 <213> ORGANISM: chimeric dengue serotype 2/4 (MVS)
 <220> FEATURE:
 <221> NAME/KEY: Xaa
 <222> LOCATION: (1226)..(1226)
 <223> OTHER INFORMATION: Arg or Lys

<400> SEQUENCE: 8

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Lys	Arg	Glu	Arg	Asn	Arg	Val	Ser	Thr	Val	Gln	Gln	Leu	Thr	Lys	Arg
								20				30			

Phe	Ser	Leu	Gly	Met	Leu	Gln	Gly	Arg	Gly	Pro	Leu	Lys	Leu	Phe	Met
								35			40		45		

Ala	Leu	Val	Ala	Phe	Leu	Arg	Phe	Leu	Thr	Ile	Pro	Pro	Thr	Ala	Gly
								50		55		60			

Ile	Leu	Lys	Arg	Trp	Gly	Thr	Ile	Lys	Ser	Lys	Ala	Ile	Asn	Val
							65	70		75		80		

Leu	Arg	Gly	Phe	Arg	Lys	Glu	Ile	Gly	Arg	Met	Leu	Asn	Ile	Leu	Asn
							85	90		95					

Arg	Arg	Arg	Ser	Ser	Ala	Gly	Met	Ile	Ile	Met	Leu	Ile	Pro	Thr	Val
							100	105		110					

Met	Ala	Phe	His	Leu	Thr	Thr	Arg	Asp	Gly	Glu	Pro	Leu	Met	Ile	Val
							115	120		125					

Ala	Lys	His	Glu	Arg	Gly	Pro	Leu	Leu	Phe	Lys	Thr	Thr	Glu	Gly
							130	135		140				

Ile	Asn	Lys	Cys	Thr	Leu	Ile	Ala	Met	Asp	Leu	Gly	Glu	Met	Cys	Glu
							145	150		155		160			

Asp	Thr	Val	Thr	Tyr	Lys	Cys	Pro	Leu	Leu	Val	Asn	Thr	Glu	Pro	Glu
							165	170		175					

Asp	Ile	Asp	Cys	Trp	Cys	Asn	Leu	Thr	Ser	Thr	Trp	Val	Met	Tyr	Gly
							180	185		190					

Thr	Cys	Thr	Gln	Ser	Gly	Glu	Arg	Arg	Glu	Lys	Arg	Ser	Val	Ala
							195	200		205				

Leu	Thr	Pro	His	Ser	Gly	Met	Gly	Leu	Glu	Thr	Arg	Ala	Glu	Thr	Trp
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 245 250 255
 Met Ile Gly Gln Thr Gly Ile Gln Arg Thr Val Phe Phe Val Leu Met
 260 265 270
 Met Leu Val Ala Pro Ser Tyr Gly Met Arg Cys Val Gly Val Gly Asn
 275 280 285
 Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ala Trp Val Asp Leu Val
 290 295 300
 Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Gln Gly Lys Pro Thr
 305 310 315 320
 Leu Asp Phe Glu Leu Thr Lys Thr Ala Lys Glu Val Ala Leu Leu
 325 330 335
 Arg Thr Tyr Cys Ile Glu Ala Ser Ile Ser Asn Ile Thr Thr Ala Thr
 340 345 350
 Arg Cys Pro Thr Gln Gly Glu Pro Tyr Leu Lys Glu Glu Gln Asp Gln
 355 360 365
 Gln Tyr Ile Cys Arg Arg Asp Val Val Asp Arg Gly Trp Gly Asn Gly
 370 375 380
 Cys Gly Leu Phe Gly Lys Gly Val Val Thr Cys Ala Lys Phe Ser
 385 390 395 400
 Cys Ser Gly Lys Ile Thr Gly Asn Leu Val Gln Ile Glu Asn Leu Glu
 405 410 415
 Tyr Thr Val Val Val Thr Val His Asn Gly Asp Thr His Ala Val Gly
 420 425 430
 Asn Asp Thr Ser Asn His Gly Val Thr Ala Thr Ile Thr Pro Arg Ser
 435 440 445
 Pro Ser Val Glu Val Lys Leu Pro Asp Tyr Gly Glu Leu Thr Leu Asp
 450 455 460
 Cys Glu Pro Arg Ser Gly Ile Asp Phe Asn Glu Met Ile Leu Met Lys
 465 470 475 480
 Met Lys Lys Thr Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu
 485 490 495
 Pro Leu Pro Trp Thr Ala Gly Ala Asp Thr Ser Glu Val His Trp Asn
 500 505 510
 Tyr Lys Glu Arg Met Val Thr Phe Lys Val Pro His Ala Lys Arg Gln
 515 520 525
 Asp Val Thr Val Leu Gly Ser Gln Glu Gly Ala Met His Ser Ala Leu
 530 535 540
 Ala Gly Ala Thr Glu Val Asp Ser Gly Asp Gly Asn His Met Phe Ala
 545 550 555 560
 Gly His Leu Lys Cys Lys Val Arg Met Glu Lys Leu Arg Ile Lys Gly
 565 570 575
 Met Ser Tyr Thr Met Cys Ser Gly Lys Phe Ser Ile Asp Lys Glu Met
 580 585 590
 Ala Glu Thr Gln His Gly Thr Thr Val Val Lys Val Lys Tyr Glu Gly
 595 600 605
 Ala Gly Ala Pro Cys Lys Val Pro Ile Glu Ile Arg Asp Val Asn Lys
 610 615 620
 Glu Lys Val Val Gly Arg Ile Ile Ser Ser Thr Pro Leu Ala Glu Asn
 625 630 635 640

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 645 650 655
 Tyr Ile Val Ile Gly Val Gly Asn Ser Ala Leu Thr Leu His Trp Phe
 660 665 670
 Arg Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ser Thr Tyr Arg Gly
 675 680 685
 Ala Lys Arg Met Ala Ile Leu Gly Glu Thr Ala Trp Asp Phe Gly Ser
 690 695 700
 Val Gly Gly Leu Phe Thr Ser Leu Gly Lys Ala Val His Gln Val Phe
 705 710 715 720
 Gly Ser Val Tyr Thr Thr Leu Phe Gly Gly Val Ser Trp Met Ile Arg
 725 730 735
 Ile Leu Ile Gly Phe Leu Val Leu Trp Ile Gly Thr Asn Ser Arg Asn
 740 745 750
 Thr Ser Met Ala Met Thr Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr
 755 760 765
 Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
 770 775 780
 Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
 785 790 795 800
 His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
 805 810 815
 Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
 820 825 830
 Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
 835 840 845
 Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
 850 855 860
 Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
 865 870 875 880
 Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
 885 890 895
 Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
 900 905 910
 Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
 915 920 925
 Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
 930 935 940
 Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
 945 950 955 960
 Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
 965 970 975
 Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
 980 985 990
 Ile Glu Val Lys Asn Cys His Trp Pro Lys Ser His Thr Leu Trp Ser
 995 1000 1005
 Asn Gly Val Leu Glu Ser Glu Met Ile Ile Pro Lys Asn Leu Ala
 1010 1015 1020
 Gly Pro Val Ser Gln His Asn Tyr Arg Pro Gly Tyr His Thr Gln
 1025 1030 1035
 Ile Thr Gly Pro Trp His Leu Gly Lys Leu Glu Met Asp Phe Asp
 1040 1045 1050
 Phe Cys Asp Gly Thr Thr Val Val Val Thr Glu Asp Cys Gly Asn

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1070	1075	1080
Thr Glu Trp Cys Cys Arg Ser Cys Thr Leu Pro Pro Leu Arg Tyr		
1085	1090	1095
Arg Gly Glu Asp Gly Cys Trp Tyr Gly Met Glu Ile Arg Pro Leu		
1100	1105	1110
Lys Glu Lys Glu Glu Asn Leu Val Asn Ser Leu Val Thr Ala Gly		
1115	1120	1125
His Gly Gln Val Asp Asn Phe Ser Leu Gly Val Leu Gly Met Ala		
1130	1135	1140
Leu Phe Leu Glu Glu Met Leu Arg Thr Arg Val Gly Thr Lys His		
1145	1150	1155
Ala Ile Leu Leu Val Ala Val Ser Phe Val Thr Leu Ile Thr Gly		
1160	1165	1170
Asn Met Ser Phe Arg Asp Leu Gly Arg Val Met Val Met Val Gly		
1175	1180	1185
Ala Thr Met Thr Gly Asp Ile Gly Met Gly Val Thr Tyr Leu Ala		
1190	1195	1200
Leu Leu Ala Ala Phe Lys Val Arg Pro Thr Phe Ala Ala Gly Leu		
1205	1210	1215
Leu Leu Arg Lys Leu Thr Ser Xaa Glu Leu Met Met Thr Thr Ile		
1220	1225	1230
Gly Ile Val Leu Leu Ser Gln Ser Thr Ile Pro Glu Thr Ile Leu		
1235	1240	1245
Glu Leu Thr Asp Ala Leu Ala Leu Gly Met Met Val Leu Lys Met		
1250	1255	1260
Val Arg Asn Met Glu Lys Tyr Gln Leu Ala Val Thr Ile Met Ala		
1265	1270	1275
Ile Leu Cys Val Pro Asn Ala Val Ile Leu Gln Asn Ala Trp Lys		
1280	1285	1290
Val Ser Cys Thr Ile Leu Ala Val Val Ser Val Ser Pro Leu Phe		
1295	1300	1305
Leu Thr Ser Ser Gln Gln Lys Thr Asp Trp Ile Pro Leu Ala Leu		
1310	1315	1320
Thr Ile Lys Gly Leu Asn Pro Thr Ala Ile Phe Leu Thr Thr Leu		
1325	1330	1335
Ser Arg Thr Ser Lys Lys Arg Ser Trp Pro Leu Asn Glu Ala Ile		
1340	1345	1350
Met Ala Val Gly Met Val Ser Ile Leu Ala Ser Ser Leu Leu Lys		
1355	1360	1365
Asn Asp Ile Pro Met Thr Gly Pro Leu Val Ala Gly Gly Leu Leu		
1370	1375	1380
Thr Val Cys Tyr Val Leu Thr Gly Arg Ser Ala Asp Leu Glu Leu		
1385	1390	1395
Glu Arg Ala Ala Asp Val Lys Trp Glu Asp Gln Ala Glu Ile Ser		
1400	1405	1410
Gly Ser Ser Pro Ile Leu Ser Ile Thr Ile Ser Glu Asp Gly Ser		
1415	1420	1425
Met Ser Ile Lys Asn Glu Glu Glu Glu Gln Thr Leu Thr Ile Leu		
1430	1435	1440
Ile Arg Thr Gly Leu Leu Val Ile Ser Gly Leu Phe Pro Val Ser		
1445	1450	1455

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Ile Pro Ile Thr Ala Ala Ala Trp Tyr Leu Trp Glu Val Lys Lys
1460 1465 1470

Gln Arg Ala Gly Val Leu Trp Asp Val Pro Ser Pro Pro Pro Met
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Gly Lys Ala Glu Leu Glu Asp Gly Ala Tyr Arg Ile Lys Gln Lys
1490 1495 1500

Gly Ile Leu Gly Tyr Ser Gln Ile Gly Ala Gly Val Tyr Lys Glu
1505 1510 1515

Gly Thr Phe His Thr Met Trp His Val Thr Arg Gly Ala Val Leu
1520 1525 1530

Met His Lys Gly Lys Arg Ile Glu Pro Ser Trp Ala Asp Val Lys
1535 1540 1545

Lys Asp Leu Ile Ser Tyr Gly Gly Gly Trp Lys Leu Glu Gly Glu
1550 1555 1560

Trp Lys Glu Gly Glu Glu Val Gln Val Leu Ala Leu Glu Pro Gly
1565 1570 1575

Lys Asn Pro Arg Ala Val Gln Thr Lys Pro Gly Leu Phe Lys Thr
1580 1585 1590

Asn Ala Gly Thr Ile Gly Ala Val Ser Leu Asp Phe Ser Pro Gly
1595 1600 1605

Thr Ser Gly Ser Pro Ile Ile Asp Lys Lys Gly Lys Val Val Gly
1610 1615 1620

Leu Tyr Gly Asn Gly Val Val Thr Arg Ser Gly Ala Tyr Val Ser
1625 1630 1635

Ala Ile Ala Gln Thr Glu Lys Ser Ile Glu Asp Asn Pro Glu Ile
1640 1645 1650

Glu Asp Asp Ile Phe Arg Lys Arg Arg Leu Thr Ile Met Asp Leu
1655 1660 1665

His Pro Gly Ala Gly Lys Thr Lys Arg Tyr Leu Pro Ala Ile Val
1670 1675 1680

Arg Glu Ala Ile Lys Arg Gly Leu Arg Thr Leu Ile Leu Ala Pro
1685 1690 1695

Thr Arg Val Val Ala Ala Glu Met Glu Glu Ala Leu Arg Gly Leu
1700 1705 1710

Pro Ile Arg Tyr Gln Thr Pro Ala Ile Arg Ala Val His Thr Gly
1715 1720 1725

Arg Glu Ile Val Asp Leu Met Cys His Ala Thr Phe Thr Met Arg
1730 1735 1740

Leu Leu Ser Pro Val Arg Val Pro Asn Tyr Asn Leu Ile Ile Met
1745 1750 1755

Asp Glu Ala His Phe Thr Asp Pro Ala Ser Ile Ala Ala Arg Gly
1760 1765 1770

Tyr Ile Ser Thr Arg Val Glu Met Gly Glu Ala Ala Gly Ile Phe
1775 1780 1785

Met Thr Ala Thr Pro Pro Gly Ser Arg Asp Pro Phe Pro Gln Ser
1790 1795 1800

Asn Ala Pro Ile Ile Asp Glu Glu Arg Glu Ile Pro Glu Arg Ser
1805 1810 1815

Trp Asn Ser Gly His Glu Trp Val Thr Asp Phe Lys Gly Lys Thr
1820 1825 1830

Val Trp Phe Val Pro Ser Ile Lys Ala Gly Asn Asp Ile Ala Ala
1835 1840 1845

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Cys Leu Arg Lys Asn Gly Lys Lys Val Ile Gln Leu Ser Arg Lys
1850 1855 1860

Thr Phe Asp Ser Glu Tyr Val Lys Thr Arg Thr Asn Asp Trp Asp
1865 1870 1875

Phe Val Val Thr Thr Asp Ile Ser Glu Met Gly Ala Asn Phe Lys
1880 1885 1890

Ala Glu Arg Val Ile Asp Pro Arg Arg Cys Met Lys Pro Val Ile
1895 1900 1905

Leu Thr Asp Gly Glu Glu Arg Val Ile Leu Ala Gly Pro Met Pro
1910 1915 1920

Val Thr His Ser Ser Ala Ala Gln Arg Arg Gly Arg Ile Gly Arg
1925 1930 1935

Asn Pro Lys Asn Glu Asn Asp Gln Tyr Ile Tyr Met Gly Glu Pro
1940 1945 1950

Leu Glu Asn Asp Glu Asp Cys Ala His Trp Lys Glu Ala Lys Met
1955 1960 1965

Leu Leu Asp Asn Ile Asn Thr Pro Glu Gly Ile Ile Pro Ser Met
1970 1975 1980

Phe Glu Pro Glu Arg Glu Lys Val Asp Ala Ile Asp Gly Glu Tyr
1985 1990 1995

Arg Leu Arg Gly Glu Ala Arg Lys Thr Phe Val Asp Leu Met Arg
2000 2005 2010

Arg Gly Asp Leu Pro Val Trp Leu Ala Tyr Arg Val Ala Ala Glu
2015 2020 2025

Gly Ile Asn Tyr Ala Asp Arg Arg Trp Cys Phe Asp Gly Val Lys
2030 2035 2040

Asn Asn Gln Ile Leu Glu Glu Asn Val Glu Val Glu Ile Trp Thr
2045 2050 2055

Lys Glu Gly Glu Arg Lys Lys Leu Lys Pro Arg Trp Leu Asp Ala
2060 2065 2070

Arg Ile Tyr Ser Asp Pro Leu Ala Leu Lys Glu Phe Lys Glu Phe
2075 2080 2085

Ala Ala Gly Arg Lys Ser Leu Thr Leu Asn Leu Ile Thr Glu Met
2090 2095 2100

Gly Arg Leu Pro Thr Phe Met Thr Gln Lys Val Arg Asp Ala Leu
2105 2110 2115

Asp Asn Leu Ala Val Leu His Thr Ala Glu Ala Gly Gly Arg Ala
2120 2125 2130

Tyr Asn His Ala Leu Ser Glu Leu Pro Glu Thr Leu Glu Thr Leu
2135 2140 2145

Leu Leu Leu Thr Leu Leu Ala Thr Val Thr Gly Gly Ile Phe Leu
2150 2155 2160

Phe Leu Met Ser Ala Arg Gly Ile Gly Lys Met Thr Leu Gly Met
2165 2170 2175

Cys Cys Ile Ile Thr Ala Ser Ile Leu Leu Trp Tyr Ala Gln Ile
2180 2185 2190

Gln Pro His Trp Ile Ala Ala Ser Ile Ile Leu Glu Phe Phe Leu
2195 2200 2205

Ile Val Leu Leu Ile Pro Glu Pro Glu Lys Gln Arg Thr Pro Gln
2210 2215 2220

Asp Asn Gln Leu Thr Tyr Val Val Ile Ala Ile Leu Thr Val Val
2225 2230 2235

Ala Ala Thr Met Ala Asn Glu Met Gly Phe Leu Glu Lys Thr Lys

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2240	2245	2250
Lys Asp Leu Gly Leu Gly Ser Ile Ala Thr Gln Gln Pro Glu Ser		
2255	2260	2265
Asn Ile Leu Asp Ile Asp Leu Arg Pro Ala Ser Ala Trp Thr Leu		
2270	2275	2280
Tyr Ala Val Ala Thr Thr Phe Val Thr Pro Met Leu Arg His Ser		
2285	2290	2295
Ile Glu Asn Ser Ser Val Asn Val Ser Leu Thr Ala Ile Ala Asn		
2300	2305	2310
Gln Ala Thr Val Leu Met Gly Leu Gly Lys Gly Trp Pro Leu Ser		
2315	2320	2325
Lys Met Asp Ile Gly Val Pro Leu Leu Ala Ile Gly Cys Tyr Ser		
2330	2335	2340
Gln Val Asn Pro Ile Thr Leu Thr Ala Ala Leu Phe Leu Leu Val		
2345	2350	2355
Ala His Tyr Ala Ile Ile Gly Pro Gly Leu Gln Ala Lys Ala Thr		
2360	2365	2370
Arg Glu Ala Gln Lys Arg Ala Ala Ala Gly Ile Met Lys Asn Pro		
2375	2380	2385
Thr Val Asp Gly Ile Thr Val Ile Asp Leu Asp Pro Ile Pro Tyr		
2390	2395	2400
Asp Pro Lys Phe Glu Lys Gln Leu Gly Gln Val Met Leu Leu Val		
2405	2410	2415
Leu Cys Val Thr Gln Val Leu Met Met Arg Thr Thr Trp Ala Leu		
2420	2425	2430
Cys Glu Ala Leu Thr Leu Ala Thr Gly Pro Ile Ser Thr Leu Trp		
2435	2440	2445
Glu Gly Asn Pro Gly Arg Phe Trp Asn Thr Thr Ile Ala Val Ser		
2450	2455	2460
Met Ala Asn Ile Phe Arg Gly Ser Tyr Leu Ala Gly Ala Gly Leu		
2465	2470	2475
Leu Phe Ser Ile Met Lys Asn Thr Thr Asn Thr Arg Arg Gly Thr		
2480	2485	2490
Gly Asn Ile Gly Glu Thr Leu Gly Glu Lys Trp Lys Ser Arg Leu		
2495	2500	2505
Asn Ala Leu Gly Lys Ser Glu Phe Gln Ile Tyr Lys Lys Ser Gly		
2510	2515	2520
Ile Gln Glu Val Asp Arg Thr Leu Ala Lys Glu Gly Ile Lys Arg		
2525	2530	2535
Gly Glu Thr Asp His His Ala Val Ser Arg Gly Ser Ala Lys Leu		
2540	2545	2550
Arg Trp Phe Val Glu Arg Asn Met Val Thr Pro Glu Gly Lys Val		
2555	2560	2565
Val Asp Leu Gly Cys Gly Arg Gly Gly Trp Ser Tyr Tyr Cys Gly		
2570	2575	2580
Gly Leu Lys Asn Val Arg Glu Val Lys Gly Leu Thr Lys Gly Gly		
2585	2590	2595
Pro Gly His Glu Glu Pro Ile Pro Met Ser Thr Tyr Gly Trp Asn		
2600	2605	2610
Leu Val Arg Leu Gln Ser Gly Val Asp Val Phe Phe Ile Pro Pro		
2615	2620	2625
Glu Lys Cys Asp Thr Leu Leu Cys Asp Ile Gly Glu Ser Ser Pro		
2630	2635	2640

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Asn	Pro	Thr	Val	Glu	Ala	Gly	Arg	Thr	Leu	Arg	Val	Leu	Asn	Leu
2645			2650			2655								
Val	Glu	Asn	Trp	Leu	Asn	Asn	Asn	Thr	Gln	Phe	Cys	Ile	Lys	Val
2660			2665				2670							
Leu	Asn	Pro	Tyr	Met	Pro	Ser	Val	Ile	Glu	Lys	Met	Glu	Ala	Leu
2675			2680				2685							
Gln	Arg	Lys	Tyr	Gly	Gly	Ala	Leu	Val	Arg	Asn	Pro	Leu	Ser	Arg
2690			2695				2700							
Asn	Ser	Thr	His	Glu	Met	Tyr	Trp	Val	Ser	Asn	Ala	Ser	Gly	Asn
2705			2710				2715							
Ile	Val	Ser	Ser	Val	Asn	Met	Ile	Ser	Arg	Met	Leu	Ile	Asn	Arg
2720			2725				2730							
Phe	Thr	Met	Arg	Tyr	Lys	Lys	Ala	Thr	Tyr	Glu	Pro	Asp	Val	Asp
2735			2740				2745							
Leu	Gly	Ser	Gly	Thr	Arg	Asn	Ile	Gly	Ile	Glu	Ser	Glu	Ile	Pro
2750			2755				2760							
Asn	Leu	Asp	Ile	Ile	Gly	Lys	Arg	Ile	Glu	Lys	Ile	Lys	Gln	Glu
2765			2770				2775							
His	Glu	Thr	Ser	Trp	His	Tyr	Asp	Gln	Asp	His	Pro	Tyr	Lys	Thr
2780			2785				2790							
Trp	Ala	Tyr	His	Gly	Ser	Tyr	Glu	Thr	Lys	Gln	Thr	Gly	Ser	Ala
2795			2800				2805							
Ser	Ser	Met	Val	Asn	Gly	Val	Val	Arg	Leu	Leu	Thr	Lys	Pro	Trp
2810			2815				2820							
Asp	Val	Val	Pro	Met	Val	Thr	Gln	Met	Ala	Met	Thr	Asp	Thr	Thr
2825			2830				2835							
Pro	Phe	Gly	Gln	Gln	Arg	Val	Phe	Lys	Glu	Lys	Val	Asp	Thr	Arg
2840			2845				2850							
Thr	Gln	Glu	Pro	Lys	Glu	Gly	Thr	Lys	Lys	Leu	Met	Lys	Ile	Thr
2855			2860				2865							
Ala	Glu	Trp	Leu	Trp	Lys	Glu	Leu	Gly	Lys	Lys	Thr	Pro	Arg	
2870			2875				2880							
Met	Cys	Thr	Arg	Glu	Glu	Phe	Thr	Arg	Lys	Val	Arg	Ser	Asn	Ala
2885			2890				2895							
Ala	Leu	Gly	Ala	Ile	Phe	Thr	Asp	Glu	Asn	Lys	Trp	Lys	Ser	Ala
2900			2905				2910							
Arg	Glu	Ala	Val	Glu	Asp	Ser	Arg	Phe	Trp	Glu	Leu	Val	Asp	Lys
2915			2920				2925							
Glu	Arg	Asn	Leu	His	Leu	Glu	Gly	Lys	Cys	Glu	Thr	Cys	Val	Tyr
2930			2935				2940							
Asn	Met	Met	Gly	Lys	Arg	Glu	Lys	Lys	Leu	Gly	Glu	Phe	Gly	Lys
2945			2950				2955							
Ala	Lys	Gly	Ser	Arg	Ala	Ile	Trp	Tyr	Met	Trp	Leu	Gly	Ala	Arg
2960			2965				2970							
Phe	Leu	Glu	Phe	Glu	Ala	Leu	Gly	Phe	Leu	Asn	Glu	Asp	His	Trp
2975			2980				2985							
Phe	Ser	Arg	Glu	Asn	Ser	Leu	Ser	Gly	Val	Glu	Gly	Glu	Gly	Leu
2990			2995				3000							
His	Lys	Leu	Gly	Tyr	Ile	Leu	Arg	Asp	Val	Ser	Lys	Lys	Glu	Gly
3005			3010				3015							
Gly	Ala	Met	Tyr	Ala	Asp	Asp	Thr	Ala	Gly	Trp	Asp	Thr	Arg	Ile
3020			3025				3030							

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Thr Leu Glu Asp Leu Lys Asn Glu Glu Met Val Thr Asn His Met
 3035 3040 3045
 Glu Gly Glu His Lys Lys Leu Ala Glu Ala Ile Phe Lys Leu Thr
 3050 3055 3060
 Tyr Gln Asn Lys Val Val Arg Val Gln Arg Pro Thr Pro Arg Gly
 3065 3070 3075
 Thr Val Met Asp Ile Ile Ser Arg Arg Asp Gln Arg Gly Ser Gly
 3080 3085 3090
 Gln Val Gly Thr Tyr Gly Leu Asn Thr Phe Thr Asn Met Glu Ala
 3095 3100 3105
 Gln Leu Ile Arg Gln Met Glu Gly Glu Gly Val Phe Lys Ser Ile
 3110 3115 3120
 Gln His Leu Thr Ile Thr Glu Glu Ile Ala Val Gln Asn Trp Leu
 3125 3130 3135
 Ala Arg Val Gly Arg Glu Arg Leu Ser Arg Met Ala Ile Ser Gly
 3140 3145 3150
 Asp Asp Cys Val Val Lys Pro Leu Asp Asp Arg Phe Ala Ser Ala
 3155 3160 3165
 Leu Thr Ala Leu Asn Asp Met Gly Lys Ile Arg Lys Asp Ile Gln
 3170 3175 3180
 Gln Trp Glu Pro Ser Arg Gly Trp Asn Asp Trp Thr Gln Val Pro
 3185 3190 3195
 Phe Cys Ser His His Phe His Glu Leu Ile Met Lys Asp Gly Arg
 3200 3205 3210
 Val Leu Val Val Pro Cys Arg Asn Gln Asp Glu Leu Ile Gly Arg
 3215 3220 3225
 Ala Arg Ile Ser Gln Gly Ala Gly Trp Ser Leu Arg Glu Thr Ala
 3230 3235 3240
 Cys Leu Gly Lys Ser Tyr Ala Gln Met Trp Ser Leu Met Tyr Phe
 3245 3250 3255
 His Arg Arg Asp Leu Arg Leu Ala Ala Asn Ala Ile Cys Ser Ala
 3260 3265 3270
 Val Pro Ser His Trp Val Pro Thr Ser Arg Thr Thr Trp Ser Ile
 3275 3280 3285
 His Ala Lys His Glu Trp Met Thr Thr Glu Asp Met Leu Thr Val
 3290 3295 3300
 Trp Asn Arg Val Trp Ile Gln Glu Asn Pro Trp Met Glu Asp Lys
 3305 3310 3315
 Thr Pro Val Glu Ser Trp Glu Glu Ile Pro Tyr Leu Gly Lys Arg
 3320 3325 3330
 Glu Asp Gln Trp Cys Gly Ser Leu Ile Gly Leu Thr Ser Arg Ala
 3335 3340 3345
 Thr Trp Ala Lys Asn Ile Gln Ala Ala Ile Asn Gln Val Arg Ser
 3350 3355 3360
 Leu Ile Gly Asn Glu Glu Tyr Thr Asp Tyr Met Pro Ser Met Lys
 3365 3370 3375
 Arg Phe Arg Arg Glu Glu Glu Ala Gly Val Leu Trp
 3380 3385 3390

The invention claimed is:

1. A method of vaccinating against virologically confirmable dengue disease in subjects aged 4 to 60 years of age, the method providing a combined vaccine efficacy of at least 60% which is represented by at least 60% reduction in dengue disease occurrence in vaccinated subjects compared

to unvaccinated subjects, in each of seropositive subjects and seronegative subjects, for at least 12 months after a second unit dose administration, by administering to a subject population of seropositive subjects, seronegative subjects, or a combination thereof, a tetravalent dengue virus composition including four dengue virus strains represent-

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ing serotype 1, serotype 2, serotype 3 and serotype 4, represented by a chimeric dengue serotype 2/1 strain, a dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain, and a chimeric dengue serotype 2/4 strain, the dengue serotype 2 strain being derived from the wild type virus strain DEN-2 16681 and differing in at least three nucleotides from the wild type as follows:

- a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)
- b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)
- c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

the three chimeric dengue strains being derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:
 a DENV-2/1 chimera,
 a DENV-2/3 chimera and
 a DENV-2/4 chimera,

the method consisting of:

selecting a subject without determining whether the subject had a previous dengue infection,

subcutaneously administering a first unit dose of said tetravalent dengue vaccine composition to said subject, the first unit dose corresponding to a dose of 0.5 mL comprising

- (i) the dengue serotype 1 with a concentration of at least 3.3 log 10 pfu/0.5 mL,
- (ii) the dengue serotype 2 with a concentration of at least 2.7 log 10 pfu/0.5 mL,
- (iii) the dengue serotype 3 with a concentration of at least 4.0 log 10 pfu/0.5 mL, and
- (iv) the dengue serotype 4 with a concentration of at least 4.5 log 10 pfu/0.5 mL,

subcutaneously administering to said subject a second unit dose of said tetravalent dengue vaccine composition within 3 months after the first unit dose, the second unit dose corresponding to a dose of 0.5 mL comprising

- (i) the dengue serotype 1 with a concentration of at least 3.3 log 10 pfu/0.5 mL,
- (ii) the dengue serotype 2 with a concentration of at least 2.7 log 10 pfu/0.5 mL,
- (iii) the dengue serotype 3 with a concentration of at least 4.0 log 10 pfu/0.5 mL, and
- (iv) the dengue serotype 4 with a concentration of at least 4.5 log 10 pfu/0.5 mL,

and optionally administering a booster dose of said tetravalent dengue vaccine composition to said subject at least 12 months after administration of the second unit dose.

2. The method of claim 1, which is safe.

3. The method of claim 1, wherein the subject is under 9 years of age, 4 to 5 years of age, 6 to 11 years of age or 12 to 16 years of age.

4. The method of claim 1, wherein the subject or subject population is from a dengue endemic region.

5. The method of claim 1, wherein the subject or subject population is from a dengue non-endemic region.

6. The method of claim 1, wherein the occurrence of vaccine related serious adverse events is less than 0.1%.

7. The method of claim 1, wherein the combined vaccine efficacy against all four dengue serotypes is measured using a 2-sided 95% confidence interval, wherein the lower bound is more than 60%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline.

8. The method of claim 1, wherein the combined vaccine efficacy against dengue serotype 2 is measured using a

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2-sided 95% confidence interval, wherein the lower bound is more than 70%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline.

9. The method of claim 1, wherein the dengue disease occurrence includes occurrences of virologically-confirmed dengue disease with hospitalization, wherein the combined vaccine efficacy against virologically-confirmed dengue dis-

ease with hospitalization against all four serotypes, is measured using a 2-sided 95% confidence interval, wherein the lower bound is more than 70%, when measured against placebo in a subject population of at least 2,000 healthy subjects being seronegative against all serotypes at baseline.

10. The method of claim 1, wherein the combined vaccine efficacy against all four dengue serotypes is measured using a 2-sided 95% confidence interval, wherein the lower bound is more than 70%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose or said placebo is administered at least twice within less than 6 months, and optionally at least 4 weeks apart, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule.

11. The method of claim 1, having a combined relative risk against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the upper bound is less than 0.50, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline.

12. The method of claim 1, having a relative risk for dengue disease with hospitalization which is 1 or less, or 0.8 or less, or 0.6 or less, when measured against placebo in a subject population of at least 1,000 healthy subjects, or at least 5,000 healthy subjects, or at least 10,000 healthy subjects irrespective of serostatus at baseline and in age groups from 4 to 16 years.

13. The method of claim 1, wherein the unit dose upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent comprises:

- (i) dengue serotype 1 with a concentration of 3.3 log 10 pfu/0.5 mL to 5.0 log 10 pfu/0.5 mL,
- (ii) dengue serotype 2 with a concentration of 2.7 log 10 pfu/0.5 mL to 4.9 log 10 pfu/0.5 mL,
- (iii) dengue serotype 3 with a concentration of 4.0 log 10 pfu/0.5 mL to 5.7 log 10 pfu/0.5 mL, and
- (iv) dengue serotype 4 with a concentration of 4.5 log 10 pfu/0.5 mL to 6.2 log 10 pfu/0.5 mL,

and optionally comprises about 15% (w/v) α,α -trehalose dihydrate, about 1% (w/v) poloxamer 407, about 0.1% (w/v) human serum albumin, and about 100 mM sodium chloride when measured in 0.5 mL.

14. The method of claim 1, wherein the first unit dose and second unit dose each are reconstituted from a lyophilized composition.

15. A method of vaccinating against virologically confirmable dengue disease with hospitalization in subjects aged 4 to 60 years of age, the method providing a combined vaccine efficacy against virologically confirmable dengue disease with hospitalization of at least 70% which is represented by a reduction of at least 70% dengue disease with hospitalization occurrence in vaccinated subjects aged 4 to 60 years compared to unvaccinated subjects, in each of seropositive subjects and seronegative subjects, for at least

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12 months after a second unit dose administration, by administering to a subject population of seropositive subjects, seronegative subjects, or a combination thereof a tetravalent dengue virus composition including four dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4, represented by a chimeric dengue serotype 2/1 strain, a dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain, and a chimeric dengue serotype 2/4 strain,

the dengue serotype 2 strain being derived from the wild type virus strain DEN-2 16681 and differing in at least three nucleotides from the wild type as follows:

- a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)
- b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)
- c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

the three chimeric dengue strains being derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera,

the method consisting of:

selecting a subject without determining whether the subject had a previous dengue infection,
subcutaneously administering a first unit dose of said tetravalent dengue vaccine composition to said subject, the first unit dose corresponding to a dose of 0.5 ml comprising

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- (i) the dengue serotype 1 with a concentration of at least $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) the dengue serotype 2 with a concentration of at least $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) the dengue serotype 3 with a concentration of at least $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) the dengue serotype 4 with a concentration of at least $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$,

subcutaneously administering to said subject a second unit dose of said tetravalent dengue vaccine composition within 3 months after the first unit dose, the second unit dose corresponding to a dose of 0.5 ml comprising

- (i) the dengue serotype 1 with a concentration of at least $3.3 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (ii) the dengue serotype 2 with a concentration of at least $2.7 \log 10 \text{ pfu}/0.5 \text{ mL}$,
- (iii) the dengue serotype 3 with a concentration of at least $4.0 \log 10 \text{ pfu}/0.5 \text{ mL}$, and
- (iv) the dengue serotype 4 with a concentration of at least $4.5 \log 10 \text{ pfu}/0.5 \text{ mL}$,

and optionally administering a booster dose of said tetravalent dengue vaccine composition to said subject at least 12 months after administration of the second unit dose.

16. The method of claim 1, wherein the combined vaccine efficacy is 70% or more.

17. The method of claim 15, wherein the combined vaccine efficacy is more than 75%.

18. The method of claim 1, wherein the subject is from 4 years old to 16 years old.

19. The method of claim 15, wherein the subject is from 4 years old to 16 years old.

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