virus has been met with significant challenges. There are four serotypes of dengue virus (DEN1, DEN2, DEN3, and DEN4) and strains of each serotype are found circulating throughout the dengue endemic regions of the world. Natural infection confers long lasting immunity to the infecting serotype but not to other dengue serotypes. The more severe forms of the disease (DHF/DSS) occur most often after secondary dengue infection, when infection with one serotype of dengue virus is followed by a second infection with another serotype. The more frequent association of DHF and DSS with secondary dengue infection has been hypothesized to be due to non-neutralizing antibodies induced by infection with one virus type enhancing infectivity of a second dengue virus type (antibody-dependent enhancement – ADE).

To date, the majority of the vaccines tested clinically are live, attenuated vaccines. The use of non-replicating vaccine candidates is also being explored. For example, Ivy *et al.* (U.S. Patent 6,432,411) disclose a tetravalent subunit vaccine comprising DEN1-4 80% E (equivalent to amino acids 1-395 of the DEN-2 envelope polypeptide) proteins. Ivy et al, *supra*, also report compositions comprising DEN 1-4 80% E and ISCOMATRIX® adjuvant. Coller *et al.* (WO 2012/154202) disclose tetravalent formulations comprising DEN1-4 80% E of DEN 1-4. Inactivated viruses may also be used as potential vaccine candidates or as components of an effective vaccine (Putnak et al. *Vaccine* 23: 4442–4452 (2005), US 6190859, US 6254873 and Sterner *et al.* WO 2007/002470).

However, despite all these efforts to develop a dengue vaccine, to date no dengue vaccine is currently registered. Thus, there remains a need for a stable, safe, and effective vaccine that can induce a protective immune response against dengue infection and/or dengue-related disease.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~ disclosure relates to a dengue virus vaccine composition comprising a live, attenuated dengue vaccine ("LAV") and a second dengue vaccine, wherein the second dengue vaccine is a non-replicating dengue vaccine. In some embodiments, the non-replicating dengue vaccine is selected from a recombinant dengue subunit vaccine, a DNA vaccine, a conjugate vaccine, or an inactivated dengue vaccine, wherein the live attenuated dengue vaccine comprises at least one live, attenuated dengue virus or a live, attenuated chimeric dengue virus. In one aspect, the present disclosure relates to a dengue virus vaccine composition comprising a first and a second dengue vaccine, wherein the first dengue vaccine is a live, attenuated dengue vaccine and the second dengue vaccine is a non-replicating dengue vaccine; wherein the live attenuated dengue vaccine comprises at least one live, attenuated dengue virus or at least one live attenuated chimeric flavivirus. In further embodiments, the non-replicating vaccine is either a dengue subunit vaccine or an inactivated dengue vaccine. In some embodiments of the disclosure~~invention~~, the LAV is tetravalent (i.e. comprises live, attenuated

dengue viruses from DEN 1-4, or chimeric live, attenuated dengue viruses from DEN1-4, or a combination thereof). In some embodiments of the disclosure~~invention~~, the second vaccine is a recombinant subunit vaccine which comprises at least one dengue envelope (E) protein or fragment thereof. In preferred embodiments of the disclosure~~invention~~, the dengue subunit vaccine is tetravalent and comprises truncated dengue E proteins which each consist of about

80% of the length of wild type E of DEN1, DEN2, DEN3 and DEN4, starting from amino acid residue 1 at its N-terminus. The presence of the second dengue vaccine in the same composition as the live attenuated vaccine was found not to significantly impact live virus viability, thereby permitting the live and non-replicating vaccine to be administered in the same formulation

In additional preferred embodiments of the disclosure~~invention~~, the live attenuated dengue vaccine is tetravalent and comprises four chimeric flaviviruses; wherein each of the four chimeric flaviviruses comprises the prM and E proteins of a single dengue virus serotype and the capsid and non-structural proteins of a different flavivirus, wherein each of the chimeric flaviviruses are attenuated. In some embodiments of the disclosure~~invention~~, the capsid and nonstructural proteins of the four chimeric flaviviruses are from yellow fever virus. In alternative preferred embodiments, the capsid and nonstructural proteins of each of the four chimeric flaviviruses are from a different dengue serotype than the prM and E proteins.

The disclosure ~~invention~~ also relates to dengue virus vaccine compositions that comprise a live, attenuated dengue vaccine and a recombinant dengue subunit vaccine and an adjuvant. In some embodiments described herein, the adjuvant is an aluminum salt adjuvant. In alternative embodiments, the adjuvant is a saponin-based adjuvant or a toll-like receptor agonist adjuvant.

~~Other aspects of this~~ The disclosure also ~~invention~~ include methods of preventing dengue infection, or preventing or ameliorating the symptoms thereof, comprising administering an effective amount of the dengue virus vaccine compositions of the disclosure ~~invention~~ to a subject. In additional embodiments of this aspect of the disclosure~~invention~~, the compositions are administered in a prime/boost regime, wherein a dose of the composition is administered to a patient, a predetermined amount of time is allowed to pass, and a second dose of the composition is administered to the patient a second time. Additional doses may optionally be administered to the patient after a predetermined amount of time has passed between each dose.

In another aspect, the present disclosure relates to use of a a dengue virus vaccine composition in the manufacture of a medicament for the prevention of dengue infection, or the prevention or amelioration of the symptoms thereof, wherein the dengue virus vaccine composition comprises a mixture of a first and a second dengue vaccine, wherein the first dengue vaccine is a live, attenuated dengue vaccine and the second dengue vaccine is a non-replicating dengue vaccine,

In additional embodiments, the disclosure ~~invention~~ relates to a method of inducing an immune response against dengue infection, thereby reducing the likelihood of dengue infection, comprising the steps of: (a) mixing a first vaccine comprising a live, attenuated, tetravalent dengue vaccine and a second vaccine comprising a recombinant tetravalent dengue subunit vaccine or an inactivated dengue vaccine; wherein the live, attenuated, tetravalent dengue vaccine comprises live attenuated dengue virus or live attenuated chimeric dengue virus of dengue serotypes 1-4; and wherein the recombinant tetravalent dengue subunit

vaccine comprises dengue envelope (E) proteins of dengue serotypes 1-4 or fragments thereof, to form a dengue virus vaccine composition; (b) administering the dengue virus vaccine composition of step (a) after mixing to a patient in which an immune response against dengue is to be induced, thereby reducing the likelihood of dengue infection. In some embodiments of the method, the immune response prevents dengue infection or prevents or ameliorates the symptoms thereof. In additional embodiments of ~~this aspect of~~ the disclosure~~invention~~, a second dose of the composition is administered to the patient after a predetermined amount of time is allowed to pass. The first

vaccine and the second vaccine of the second dose of the composition can be formulated in a single vial or in two separate vials and mixed together prior to administration to the patient.

In another aspect, the disclosure relates to use of a first dengue virus vaccine composition as defined herein and a second dengue virus vaccine composition as defined herein in the manufacture of a medicament for the prevention or reduction of the likelihood of dengue infection in a patient in need thereof, wherein the second dengue virus vaccine composition is to be administered to the patient after a predetermined amount of time had passed after the first dengue virus vaccine composition had been administered to the patient.

The disclosure ~~invention~~ also relates to the use of the dengue virus vaccine compositions of the invention for the treatment or prophylaxis of disease associated with dengue infection, such as dengue fever, DSS or DHF.

In another aspect, the disclosure relates to use of the dengue virus vaccine composition as defined herein in the manufacture of a medicament for the treatment or prophylaxis of disease associated with dengue infection.

In another aspect, the disclosure relates to use of the dengue virus vaccine composition as defined herein in the manufacture of a medicament for the induction of an immune response against dengue virus in a patient.

In another aspect, the disclosure relates to a dengue virus vaccine composition as defined herein for use in the treatment or prophylaxis of disease associated with dengue infection.

As used throughout the specification and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise.

Reference to "or" indicates either or both possibilities unless the context clear dictates one of the indicated possibilities. In some cases, "and/or" was employed to highlight either or both possibilities.

As used throughout the specification and appended claims, the following definitions and abbreviations apply:

The term "live attenuated vaccine," also referred to as "LAV" herein, means a vaccine comprising an effective amount of at least one live attenuated dengue virus and/or chimeric dengue virus and a pharmaceutically acceptable carrier, wherein the ability of the virus to cause disease is reduced compared to wild-type dengue virus.

The term "chimeric flavivirus vaccine" refers to a vaccine comprising an effective amount of a virus containing prM and/or E proteins of a dengue virus serotype and the capsid and non-structural proteins of a second, different flavivirus and a pharmaceutically acceptable carrier. The second flavivirus can be a different dengue serotype or another flavivirus, such as yellow fever virus.

"Non-replicating vaccine" refers to a dengue virus vaccine for the prevention or treatment of dengue virus infection or the clinical symptoms thereof, selected from a recombinant subunit vaccine, an inactivated vaccine, a conjugate vaccine, or a DNA vaccine.

"Inactivated vaccine" refers to a vaccine comprising an effective amount of a killed or inactive whole dengue virus and a pharmaceutically acceptable carrier, wherein the virus is inactivated by any means, including with chemicals, heat or radiation. An inactivated vaccine has a low residual infectivity following inactivation, e.g. <5 plaque forming units (PFU's)/mL after inactivation. In preferred embodiments, there is very low amount of residual infectivity following inactivation, e.g. $\leq$0.4 PFU's/mL, $\leq$3 PFU's/mL, or $\leq$2 PFU's/mL, <1 PFU/mL, $\leq$0.5 PFU/mL, or $\leq$0.1 PFU/mL. The PFU's of a particular vaccine may be determined using, for example, by using a plaque assay, an immunostaining assay, or other method known in the art for detecting viral infectivity.

"Conjugate vaccine" refers to a vaccine comprising a dengue antigen covalently attached to a carrier protein.

A "DNA vaccine" is a vaccine comprising a sequence of nucleotides that encodes a dengue protein antigen, including dengue proteins, dengue protein fragments, and dengue

composition is from about 1.0 μg to about 100 μg.

12. The composition of claim 11, wherein the amount of DEN4 E protein is about 1.5 to about 2.5 times the amount of DEN1, DEN2, and DEN3 E proteins.

13. The composition of any of claims 10-12, wherein each E protein is recombinantly produced and expressed in insect host cells.

14. The composition of any of claims 1-13, wherein the live attenuated dengue vaccine is tetravalent.

15. The composition of claim 14, wherein the live attenuated dengue vaccine comprises four chimeric flaviviruses; wherein each chimeric flavivirus comprises the prM and E proteins of a single dengue virus serotype and the capsid and non-structural proteins of a different flavivirus, wherein each of the chimeric flaviviruses are attenuated.

16. The composition of claim 15, wherein the capsid and nonstructural proteins of the four chimeric flaviviruses are from yellow fever virus.

17. The composition of claim 15, wherein the capsid and nonstructural proteins of each of the four chimeric flaviviruses are from a different dengue serotype than the prM and E proteins.

18. The composition of any of claims 14- 17, wherein the potency of the live attenuated dengue vaccine is from 10 to about $1x10^7$ plaque forming units (PFU's).

19. The composition of claim 18, wherein the potency of the live attenuated dengue vaccine is from about $1x10^3$ to about $1x10^5$ PFU's.

~~20. A method of inducing an immune response against dengue in a patient in need thereof, comprising administering an effective amount of the dengue virus vaccine composition of any of claims 1-19 to the patient.~~

~~21. A method of preventing dengue infection, or preventing or ameliorating the symptoms thereof in a patient in need thereof, comprising administering an effective amount of the dengue virus vaccine composition of any of claims 1-19 to the patient.~~

~~22. A method of preventing dengue infection, or preventing or ameliorating~~

20. Use of a dengue virus vaccine composition in the manufacture of a medicament for the prevention of dengue infection, or the prevention or amelioration of the symptoms thereof, wherein the dengue virus vaccine composition comprises ~~comprising the steps of:~~

~~(a) mixing~~ a mixture of a first and a second dengue vaccine ~~to form a dengue virus vaccine composition~~, wherein the first dengue vaccine is a live, attenuated dengue vaccine and the second dengue vaccine is a non-replicating dengue vaccine~~; and~~

~~(b) administering the dengue virus vaccine composition of step (a) to a patient in which dengue infection or the symptoms thereof are to be prevented or ameliorated~~.

~~23~~21. The ~~method~~ use of claim ~~22~~20, wherein the non-replicating dengue vaccine is a recombinant dengue subunit vaccine or an inactivated dengue vaccine.

~~24~~22. The ~~method~~ use of claim ~~22~~ 20 or claim ~~23~~21, wherein the first dengue vaccine is tetravalent and comprises a DEN1, DEN2, DEN3, and DEN 4 component, wherein each component comprises either a live attenuated dengue virus or a live attenuated chimeric flavivirus.

~~25~~23. The ~~method~~ use of claim ~~23~~ 21 or ~~24~~22, wherein the second dengue vaccine is a tetravalent recombinant dengue subunit vaccine comprising dengue E proteins, or fragments thereof, from DEN1, DEN2, DEN3, and DEN4.

~~26. The method of claim 25, wherein the live, attenuated vaccine is lyophilized and the recombinant subunit vaccine is a liquid prior to mixing.~~

~~27. The method of claim 26, wherein the mixing of step (a) comprises reconstituting the lyophilized vaccine with the liquid vaccine.~~

~~28. The method of claim 26, wherein the lyophilized vaccine is reconstituted with a sterile diluent prior to mixing with the liquid vaccine in step (a).~~

~~29. The method of claim 25, wherein both the live, attenuated vaccine and the recombinant subunit vaccine are in the form of a liquid prior to mixing.~~

~~30~~24. The ~~method~~ use of ~~any of~~ claims ~~25-29~~ 23 wherein the E proteins each constitute about 80% of the length of wild type E of DEN1, DEN2, DEN3 and DEN4, starting from amino acid residue 1 at its N-terminus.

~~31~~25. The ~~method~~ use of claim ~~22~~20, wherein the live attenuated vaccine comprises four chimeric flaviviruses; wherein each of the chimeric flavivirus comprises the prM and E proteins of a single dengue virus serotype and the capsid and non-structural proteins of a

different flavivirus, wherein the each of the chimeric flavivirus is attenuated.

~~32~~26. The ~~method~~ use of claim ~~31~~25, wherein the capsid and nonstructural proteins of the four chimeric flaviviruses are from yellow fever virus.

~~33~~27. The ~~method~~ use of claim ~~31~~25, wherein the capsid and nonstructural proteins of each of the four chimeric flaviviruses are from a different dengue serotype than the prM and E proteins.

~~34~~28. Use of a first dengue virus vaccine composition according to any of claims 1-19 and a second dengue virus vaccine composition according to any of claims 1-19 in the manufacture of a medicament for the prevention or reduction of ~~A method of preventing or reducing~~ the likelihood of dengue infection in a patient in need thereof, wherein the second dengue virus vaccine composition is to be administered to the patient after a predetermined amount of time had passed after the first dengue virus vaccine composition had been administered to the ~~in a~~ patient ~~in need thereof, comprising the steps of:~~

~~(a) administering a first dengue virus vaccine composition according to any of claims 1-19 to the patient;~~

~~(b) waiting for a predetermined amount of time to pass after step (a);~~

~~(c) administering to the patient a second dengue virus vaccine composition according to any of claims 1-19; whereby the dengue infection is prevented or the likelihood of being infected with dengue is reduced in the patient~~.

~~35~~29. The ~~method~~ use of claim ~~34~~28, wherein the first and second dengue virus vaccine composition are the same.

~~36~~30. The ~~method~~ use of claim ~~34~~28 or ~~35~~29, wherein the predetermined amount of time ~~in step (b)~~ is from 2 months to 2 years.

~~37. The method of any of claims 34-36, further comprising repeating steps (b) and (c) one or more times.~~

~~38~~31. The ~~method~~ use of any of claims ~~34~~28-~~36~~30, wherein the first and second dengue virus vaccine compositions are to be administered by intramuscular or subcutaneous injection.

~~39~~32. Use of the dengue virus vaccine composition of any of claims 1-19 in the manufacture of a medicament for the treatment or prophylaxis of disease associated with dengue infection.

~~40~~33. Use of the dengue virus vaccine composition of any of claims 1-19 in the manufacture of a medicament for the induction of an immune response against dengue virus in a patient.

~~41~~34. A dengue virus vaccine composition according to any of claims 1-19 for use in the treatment or prophylaxis of disease associated with dengue infection.