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 (the peptide region of DEN1-4 corresponding to amino acids 1-395 of the DENV-2 envelope polypeptide) proteins. Ivy et al, *supra*, also report compositions comprising DENV 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). Compositions comprising a live attenuated dengue virus vaccine and a non-replicating dengue vaccine are disclosed in International Patent Application No. PCT/US14/042625 (WO/2014/204892).

However, despite prior 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

The present disclosure relates to a dengue virus vaccine composition comprising a pharmaceutically effective amount of (a) a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus (LACV), wherein the LAV and the LACV comprise a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated (UTR) region; which reduces the replicative capacity of the virus, and (b) a non-replicating dengue vaccine.

In one aspect, the disclosure relates to a dengue virus vaccine composition comprising a pharmaceutically effective amount of: (a) a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus (LACV), wherein the LAV and the LACV comprise a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated region (UTR), wherein the live attenuated dengue vaccine comprises a LAV that is immunogenic against dengue serotype 3, wherein the viral genome of the LAV further contains a deletion of nucleotides upstream from the Δ30 deletion corresponding to the TL-3 structure of

the 3' UTR; and (b) a tetravalent dengue subunit vaccine which comprises dengue E proteins from dengue virus type 1 (DEN1), dengue virus type 2 (DEN2), dengue virus type 3 (DEN3), and dengue virus type 4 (DEN4), wherein each of the E proteins constitute about 80% of the length of wild type E proteins of DEN1, DEN2, DEN3, and DEN4, respectively, starting from amino acid residue 1 at its N-terminus.

The presence of the non-replicating dengue vaccine in the same composition as the live attenuated vaccine was found to not significantly impact live virus viability, thereby permitting the live and non-replicating vaccine to be administered in the same formulation ("co-formulation").

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

(a) mixing a first dengue vaccine and a second dengue vaccine, to form a dengue virus vaccine composition,

wherein the first dengue vaccine is a live attenuated tetravalent dengue vaccine comprising live attenuated viruses (LAV) of dengue serotypes 1-4, or live attenuated chimeric flaviviruses (LACV) that are immunogenic against dengue serotypes 1-4, or a combination thereof; wherein at least one LAV or LACV is a Δ30LAV or a Δ30LACV;

wherein the second dengue vaccine is a recombinant tetravalent dengue subunit vaccine or an inactivated tetravalent dengue vaccine; and wherein the recombinant tetravalent dengue subunit vaccine comprises dengue envelope (E) proteins of dengue serotypes 1-4 or fragments thereof;

(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 of the disclosure, the immune response prevents dengue infection or prevents or ameliorates the symptoms thereof. In additional embodiments of the disclosure, a second dose of the composition is administered to the patient after a predetermined amount of time is allowed to pass. The first dengue vaccine and the second dengue 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.

The disclosurealso 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 induction of an immune response against dengue in a patient.

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 reduction in the likelihood of dengue infection, or prevention or amelioration of the symptoms of dengue infection, in a patient.

In another aspect, the disclosure relates to use of a dengue virus vaccine composition in the manufacture of a medicament for the reduction in the likelihood of dengue infection, or prevention or amelioration in the symptoms of dengue infection, 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 comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus (LACV), wherein the LAV and the LACV comprise a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated (UTR) region, wherein the LAV that is immunogenic against DEN3 contains a viral genome that further comprises a deletion of nucleotides upstream from the Δ30 deletion that corresponds to the TL-3 structure of the 3' UTR; and wherein the second dengue vaccine is a tetravalent dengue subunit vaccine which comprises dengue E proteins from dengue virus type 1 (DEN1), dengue virus type 2 (DEN2), dengue virus type 3 (DEN3), and dengue

virus type 4 (DEN4), wherein each of the E proteins constitue about 80% of the length of wild type E proteins of DEN1, DEN2, DEN3, and DEN4, respectively, starting fro amino acid residue 1 at its N-terminus.

In another aspect, the disclosure relates to use of a first and second dengue virus vaccine composition as defined herein in the manufacture of a medicament for the reduction in the likelihood of dengue infection in a patient, wherein:

the first dengue virus vaccine composition as defined herein is to be administered to the patient;

a predetermined amount of time is allowed to pass after the administration of the first dengue virus vaccine composition; and

the second dengue virus vaccine composition as defined herein is then to be administered to the patient; whereby the likelihood of being infected with dengue is reduced in the patient.

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 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 clearly 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 virus," also referred to as "LAV" herein, means a live attenuated dengue virus, wherein the ability of the virus to cause disease is reduced compared to wild-type dengue virus.

The term "live attenuated chimeric virus" (alternatively "live attenuated chimeric flavivirus") or "LACV" refers to a live attenuated chimeric virus wherein the viral genome comprises a backbone of a first flavivirus (including C, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 genes) and the preMembrane (prM) and envelope (E) genes of a second flavivirus, wherein the second flavivirus is selected from DENV1, DENV2, DENV3 or DENV4. The first flavivirus can be a different dengue serotype or another flavivirus, such as yellow fever virus.

WHAT IS CLAIMED IS:

1. A dengue virus vaccine composition comprising a pharmaceutically effective amount of:

(a) a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus (LACV), wherein the LAV and the LACV comprise a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated region (UTR), wherein the live attenuated dengue vaccine comprises a LAV that is immunogenic against dengue serotype 3, wherein the viral genome of the LAV further contains a deletion of nucleotides upstream from the Δ30 deletion corresponding to the TL-3 structure of the 3' UTR; and

(b) a tetravalent dengue subunit vaccine which comprises dengue E proteins from dengue virus type 1 (DEN1), dengue virus type 2 (DEN2), dengue virus type 3 (DEN3), and dengue virus type 4 (DEN4), wherein each of the E proteins constitute about 80% of the length of wild type E proteins of DEN1, DEN2, DEN3, and DEN4, respectively, starting from amino acid residue 1 at its N-terminus.

2. The composition of claim 1, further comprising an adjuvant.

3. The composition of claim 2, wherein the adjuvant is an aluminum salt adjuvant.

4. The composition of claim 3, wherein the amount of elemental aluminum in the composition is from about 50 μg to about 1.25 mg.

5. The composition of claim 4, wherein the amount of elemental aluminum in the composition is from about 200 μg to about 850 μg.

6. The composition of claim 2, wherein the adjuvant is a saponin-based adjuvant.

7. The composition of any of claims 1-5, wherein the amount of each E

protein in the composition is from about 0.5 μg to about 500 μg.

8. The composition of claim 7, wherein the amount of each E protein in the composition is from about 1.0 μg to about 100 μg.

9. The composition of claim 8, wherein the amount of DEN4 E protein is about 1.5 to about 2.5 times the amount of each of DEN1, DEN2, and DEN3 E proteins.

10. The composition of any of claims 7-9, wherein each E protein is recombinantly produced and expressed in insect host cells.

11. The composition of any of claims 1-10, wherein the live attenuated dengue vaccine is tetravalent.

12. The composition of any of claims 1-11, wherein the live attenuated dengue vaccine comprises one LACV that is immunogenic against dengue serotype 2 and three LAVs that are immunogenic against dengue serotypes 1, 3, and 4.

13. The composition of claim 12, wherein the viral genome of the LACV comprises preMembrane (prM) and envelope (E) genes of dengue serotype 2 and capsid and non-structural genes of a different dengue serotype.

14. The composition of claim 13, wherein the different dengue serotype is dengue serotype 4.

15. The composition of any of claims 11-14, wherein the potency of the live attenuated dengue vaccine is from 10 to about $1x10^7$ plaque forming units (PFU's).

16. The composition of claim 15, wherein the potency of the live attenuated dengue vaccine is from about $1x10^3$ to about $1x10^5$ PFU's.

17. Use of the dengue virus vaccine composition of any of claims 1-16 in the manufacture of a medicament for the induction of an immune response against dengue in a patient.

18. Use of the dengue virus vaccine composition of any of claims 1-16 in the manufacture of a medicament for the reduction in the likelihood of dengue infection, or prevention or amelioration of the symptomsof dengue infection, in a patient.

19. Use of a dengue virus vaccine composition in the manufacture of a medicament for the reduction in the likelihood of dengue infection, or prevention or amelioration in the symptomsof dengue infection, 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 comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus (LACV), wherein the LAV and the LACV comprise a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated region (UTR), wherein the LAV that is immunogenic against DEN3 contains a viral genome that further comprises a deletion of nucleotides upstream from the Δ30 deletion that corresponds to the TL-3 structure of the 3' UTR; and wherein the second dengue vaccine isa tetravalent dengue subunit vaccine which comprises dengue E proteins from dengue virus type 1 (DEN1), dengue virus type 2 (DEN2), dengue virus type 3 (DEN3), and dengue virus type 4 (DEN4), wherein each of the E proteins constitue about 80% of the length of wild type E proteins of DEN1, DEN2, DEN3, and DEN4, respectively, starting fro amino acid residue 1 at its N-terminus.

20. The use of claim 19, wherein the first dengue vaccine is tetravalent and comprises a component immunogenic against dengue serotype 1 (DEN1), a component immunogenic against dengue serotype 2 (DEN2), a component immunogenic against dengue serotype 3 (DEN3), and a component immunogenic against dengue serotype 4 (DEN 4), wherein each component comprises either a live attenuated dengue virus or a live attenuated chimeric flavivirus.

21. The use of claim 19, wherein the live attenuated dengue vaccine is lyophilized and the recombinant subunit vaccine is a liquid prior to forming the mixture.

22. The use of claim 21, wherein in the mixture, the lyophilized vaccine is reconstituted with the liquid vaccine.

23. The use of claim 21, wherein the lyophilized vaccine is reconstituted with a sterile diluent prior to forming a mixture with the liquid vaccine.

24. The use of claim 19, wherein both the live, attenuated vaccine and the recombinant subunit vaccine are in the form of a liquid prior to formng the mixture.

25. The use of claim 19, wherein the live attenuated dengue vaccine comprises one LACV that is immunogenic against dengue serotype 2 and three LAVs that are immunogenic against dengue serotype 1, dengue serotype 3 and dengue serotype 4; wherein the LACV comprises a viral genome comprising a preMembrane and an envelope gene of dengue serotype 2 and non-structural and capsid genes of dengue serotype 4.

26. Use of a first and second dengue virus vaccine composition of any of claims 1-16 in the manufacture of a medicament for the reduction in the likelihood of dengue infection in a patient, wherein:

the first dengue virus vaccine composition according to any of claims 1-16 is to be administered to the patient;

a predetermined amount of time is allowed to pass after the administration of the first dengue virus vaccine composition; and

the second dengue virus vaccine composition according to any of claims 1-16 is then to be administered to the patient; whereby the likelihood of being infected with dengue is reduced in the patient.

27. The use of claim 26, wherein the first and second dengue virus vaccine composition are the same.

28. The use of claim 26 or 27, wherein the amount of time is from 2 months to 2 years.

29. The use of any of claims 26-28, wherein the administration of the second dengue virus vaccine composition is to be repeated after the passing of the predetermined amount of time one or more times.

30. The use of any of claims 26-28, wherein the first and second dengue virus vaccine compositions are to be administered by intramuscular or subcutaneous injection.

31. Use of the dengue virus vaccine composition of any of claims 1-16 in the manufacture of a medicament for the treatment or prophylaxis of disease associated with dengue infection.

32. A dengue virus vaccine composition according to any of claims 1-16 for use in the treatment or prophylaxis of disease associated with dengue infection.