

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.

5 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.

10 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. Collier *et al.* (WO 2012/154202) disclose tetravalent
15 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

20 (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.

25 SUMMARY

The present disclosure, in one aspect, 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
30 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. 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
35 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

dengue vaccine. In further embodiments, the non-replicating dengue vaccine is either a recombinant dengue subunit vaccine or an inactivated dengue vaccine. In some embodiments of the invention, the non-replicating dengue vaccine is a recombinant dengue subunit vaccine which comprises at least one dengue envelope (E) protein or fragment thereof. In preferred
5 embodiments of the invention, the recombinant 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 dengue virus ("DENV", alternatively "DEN") serotype 1, a.k.a. DENV 1, DENV 2, DENV 3 and DENV 4, starting from amino acid residue 1 at its N-terminus.

In additional embodiments of the disclosure, the live attenuated dengue vaccine is
10 a tetravalent LAV or "LATV" (i.e. comprises live attenuated dengue viruses from DENV 1-4, or live attenuated chimeric flaviviruses from DENV 1-4, as defined herein, or a combination thereof, wherein at least one of the LAVs or LACVs is a $\Delta 30$ LAV or a $\Delta 30$ LACV). In additional embodiments of the disclosure, the live attenuated dengue vaccine is tetravalent and comprises at least one chimeric flavivirus; wherein the chimeric flavivirus comprises a viral genome that
15 contains nucleotide sequences encoding the prM and E proteins of a single dengue virus serotype and nucleotide sequences encoding the capsid and non-structural proteins of a different flavivirus, wherein the chimeric flavivirus is attenuated. In some embodiments of the disclosure, the capsid and nonstructural proteins of the chimeric flavivirus is from a different dengue serotype than the prM and E proteins.

In a preferred embodiments of the disclosure, the live attenuated dengue vaccine
20 is a live attenuated tetravalent vaccine comprising a DEN1 $\Delta 30$ virus, a DEN2/4 $\Delta 30$ virus (a DEN2 $\Delta 30$ LACV on a DEN4 backbone), a DEN3 $\Delta 30$ virus and a DEN4 $\Delta 30$ virus. See Figure 1.

The disclosure also relates to dengue virus vaccine compositions that comprise a
25 live attenuated dengue vaccine, a recombinant dengue subunit vaccine and an adjuvant, wherein the live attenuated dengue vaccine comprises at least one $\Delta 30$ LAV or $\Delta 30$ LACV. 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.

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

In additional embodiments, the disclosure 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 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 $\Delta 30$ LAV or a $\Delta 30$ LACV;

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 disclosure 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 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 thereof, 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 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 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; and wherein the second dengue vaccine is a non-replicating dengue vaccine.

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
5 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
10 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
15 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
25 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
30 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.

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

11. The composition of claim 10, wherein the amount of each E protein in the 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 any of claims 1-14, wherein the live attenuated dengue vaccine comprises one LACV that is immunogenic against dengue type 2 and three LAVs that are immunogenic against dengue types 1, 3, and 4.

16. The composition of claim 15, 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.

17. The composition of claim 16, wherein the different dengue serotype is dengue serotype 4.

18. The composition of any of claims 1-17, 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.

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

20. The composition of claim 19, wherein the potency of the live attenuated dengue vaccine is from about 1×10^3 to about 1×10^5 PFU's.

21. Use of the dengue virus vaccine composition of any of claims 1-20 in the manufacture of a medicament for the induction of an immune response against dengue in a patient.

22. Use of the dengue virus vaccine composition of any of claims 1-20 in the manufacture of a medicament for the reduction in the likelihood of dengue infection, or prevention or amelioration of the symptoms thereof, in a patient.

23. 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 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 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; and wherein the second dengue vaccine is a non-replicating dengue vaccine.

24. The use of claim 23, wherein the non-replicating dengue vaccine is a recombinant dengue subunit vaccine or an inactivated dengue vaccine.

25. The use of claim 23 or claim 24, 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.

26. The use of claim 24 or 25, 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.

27. The use of claim 26, wherein the live attenuated dengue vaccine is lyophilized and the recombinant subunit vaccine is a liquid prior to forming the mixture.

28. The use of claim 27, wherein in the mixture, the lyophilized vaccine is reconstituted with the liquid vaccine.

29. The use of claim 27, wherein the lyophilized vaccine is reconstituted with a sterile diluent prior to forming a mixture with the liquid vaccine.

30. The use of claim 26, wherein both the live, attenuated vaccine and the recombinant subunit vaccine are in the form of a liquid prior to forming the mixture.

31. The use of any of claims 26-30 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.

32. The use of claim 23, 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.

33. The use of claim 32, wherein the LAV that is immunogenic against DEN3 contains a viral genome that further comprises a deletion of nucleotides upstream from the $\Delta 30$ deletion that corresponds to the TL-3 structure of the 3' UTR.

34. Use of a first and second dengue virus vaccine composition of any of claims 1-20 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 1-20 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 1-20 is then to be administered to the patient; whereby the likelihood of being infected with dengue is reduced in the patient.

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

36. The use of claim 34 or 35, wherein the amount of time is from 2 months to 2 years.

37. The use of any of claims 34-36, further comprising repeating the administration of the second dengue virus vaccine composition after the passing of the predetermined amount of time one or more times.

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

5 39. Use of the dengue virus vaccine composition of any of claims 1-20 in the manufacture of a medicament for the treatment or prophylaxis of disease associated with dengue infection.

40. A dengue virus vaccine composition according to any of claims 1-20 for use in the treatment or prophylaxis of disease associated with dengue infection.

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