virus in the child or young adult from 2 to 17 years of age or in the child from 1 to 11 years of age, and wherein a concentration of the live attenuated dengue-2 virus serotype in the composition is at least one half a log plaque forming unit (PFU) lower than the log PFU of more than one of other dengue virus serotypes or a concentration of the dengue-2/3 chimera is at least one-half a log greater in terms of PFUs than the live attenuated dengue-2 virus serotype in the tetravalent vaccine formulation, wherein the dengue-2 virus is in the form of the of the DENV-2 16681 derived DEN-2 PDK-53 variant with a triple mutation at NS1-53, at 5'NC-57 and at NS3-250 such that the amino acid position 250 of the NS3 protein contains a valine residue, and wherein the chimeras have said DEN-2 PDK-53 genome as viral backbone and one or more structural protein genes encoding capsid, premembrane/membrane or envelope of said DEN-2 PDK-53 genome or combinations thereof replaced with one or more corresponding structural protein genes from DEN-1, DEN-3 or DEN-4.

[0007] In certain examples, chimeric dengue virus constructs of dengue virus serotypes of use in vaccines can include passage 8 (P8) live, attenuated viruses or chimeric viruses having nucleic acid sequences identified by SEQ ID NOS: 1, 3, 5 and 7 or polypeptide sequences indicated by SEQ ID NOS: 2, 4, 6 and 8. It is contemplated herein that any of the passages for any of the live, attenuated viruses described herein can be used in an immunogenic composition to induce immune responses to the represented dengue virus serotype (*e.g.*, serotypes 1-4). In accordance with these examples, an immunogenic composition that includes a P-8 isolated live, attenuated virus can be administered to a subject to induce an immunogenic response against one or more dengue virus serotypes depending on the construct selected. In addition, a live, attenuated virus can be combined with one or more of these chimeric viruses. This is contemplated for each of the live, attenuated viruses isolated/produced in each subsequent cell passages (*e.g.*, African Green Monkey Vero cell production, hereinafter: Vero cells or other appropriate cell line). It is contemplated herein that any cell line (*e.g.*, GMP approved) capable of producing dengue viruses is of use to passage any of the viral constructs at a manufacturing scale or as appropriate contemplated herein for subsequent use in a vaccine or immunogenic composition against Dengue virus.

[0008] In other examples, compositions contemplated herein can be combined with other immunogenic compositions against other Flaviviruses such as Zika virus, West Nile virus, Japanese encephalitis, St. Louis encephalitis virus, yellow fever virus or any other flavivirus

chimeric construct and/or live, attenuated virus. In certain examples, a single composition can be used against multiple flaviviruses. In some examples, compositions disclosed herein may be able to induce an immune response against Zika virus even if a vaccine composition is limited to dengue virus constructs and live, attenuated dengue virus.

What is claimed:

1. A pharmaceutical composition comprising a tetravalent vaccine formulation including all four dengue virus serotypes including a live, attenuated dengue-2 virus and dengue-2/1, dengue-2/3, and dengue-2/4 chimeras,

wherein the dengue-2 virus is in the form of the DENV-2 16681 derived DEN-2 PDK-53 variant with a triple mutation at NS1-53, at 5'NC-57 and at NS3-250 such that the amino acid position 250 of the NS3 protein contains a valine residue, and

wherein the chimeras have said DEN-2 PDK-53 genome as viral backbone and one or more structural protein genes encoding capsid, premembrane/membrane or envelope of said DEN-2 PDK-53 genome or combinations thereof replaced with one or more corresponding structural protein genes from DEN-1, DEN-3 or DEN-4, and

wherein

a concentration of the live attenuated dengue-2 virus serotype in the composition is at least one half a log plaque forming unit (PFU) lower than the log PFU of more than one of other dengue virus serotypes or

a concentration of the dengue-2/3 chimera is at least one half a log greater in terms of PFUs than the dengue-2 virus serotype in the tetravalent vaccine formulation.

2. Use of a pharmaceutical composition comprising a tetravalent vaccine formulation including all four dengue virus serotypes including a live, attenuated dengue-2 virus and dengue-2/1, dengue-2/3, and dengue-2/4 chimeras,

in the manufacture of a medicament for treating children or young adults from 2 to 17 years of age or children from 1 to 11 years of age against dengue virus infection comprising:

that the composition is to be administered subcutaneously to the child or young adult from 2 to 17 years of age or to the child from 1 to 11 years of age,

wherein the pharmaceutical composition to be administered consists of administration of a first dose of the pharmaceutical composition on day 0 and a second dose of the same pharmaceutical composition against dengue virus within 90 days of the first administration,

wherein the composition induces an immune response to dengue virus in the child or young adult from 2 to 17 years of age or in the child from 1 to 11 years of age, and

wherein

a concentration of the live attenuated dengue-2 virus serotype in the composition is at least one half a log plaque forming unit (PFU) lower than the log PFU of more than one of other dengue virus serotypes or

a concentration of the dengue-2/3 chimera is at least one-half a log greater in terms of PFUs than the live attenuated dengue-2 virus serotype in the tetravalent vaccine formulation,

wherein the dengue-2 virus is in the form of the of the DENV-2 16681 derived DEN-2 PDK-53 variant with a triple mutation at NS1-53, at 5'NC-57 and at NS3-250 such that the amino acid position 250 of the NS3 protein contains a valine residue, and

wherein the chimeras have said DEN-2 PDK-53 genome as viral backbone and one or more structural protein genes encoding capsid, premembrane/membrane or envelope of said DEN-2 PDK-53 genome or combinations thereof replaced with one or more corresponding structural protein genes from DEN-1, DEN-3 or DEN-4.

3. The pharmaceutical composition according to claim 1, or the use according to claim 2,

wherein the dengue-2/1 chimera has two further mutations such that the amino acid position 116 of the NS2A protein contains a leucine residue and the amino acid position 92 of the NS2B protein contains an aspartic acid residue,

wherein the dengue-2 virus has two further mutations such that the amino acid position 52 of the prM protein contains a glutamic acid residue and the amino acid position 412 of the NS5 protein contains a valine residue,

wherein the dengue-2/3 chimera has said DEN-2 PDK-53 genome as viral backbone and has the prM-E gene (nt-457 to -2373) of said DEN-2 PDK-53 genome replaced with the corresponding prM-E gene from wild-type DEN-3 16562 and wherein said dengue-2/3 chimera has one further mutation at said corresponding prM-E gene from wild-type DEN-3 16562 such that the amino acid position 223 of the E protein contains a serine residue, and

wherein the dengue-2/4 chimera has two further mutations such that the amino acid position 66 of the NS2A protein contains a glycine residue and the amino acid position 21 of the NS4A protein contains a valine residue and wherein the dengue-2/4 chimera is a mixed genotype with respect to amino acid position 99 of the NS2A protein containing an arginine or a lysine residue.

4. The pharmaceutical composition according to claim 1 or 3, or the use according to claim 2 or 3,

wherein the dengue-2/1 chimera has said DEN-2 PDK-53 genome as viral backbone and has the prM-E gene (nt-457 to -2379) of said DEN-2 PDK-53 genome replaced with the corresponding prM-E gene from wild-type DEN-1 16007,

wherein the dengue-2/3 chimera has said DEN-2 PDK-53 genome as viral backbone and has the prM-E gene (nt-457 to -2373) of said DEN-2 PDK-53 genome replaced with the corresponding prM-E gene from wild-type DEN-3 16562, and

wherein the dengue-2/4 chimera has said DEN-2 PDK-53 genome as viral backbone and has the prM-E gene (nt-457 to -2379) of said DEN-2 PDK-53 replaced with the corresponding prM-E gene from wild-type DEN-4 1036.

5. The pharmaceutical composition according to any one of claims 1, 3 or 4, or the use according to any one of claims 2 to 4,

wherein the ratio of the dengue-2/1 chimera, to the live, attenuated dengue-2 virus, to the dengue-2/3 chimera, to the dengue-2/4 chimera is 4:4:5:5 plaque forming units (PFU).

6. The pharmaceutical composition according to any one of claims 1, 3 or 4, or the use according to any one of claims 2 to 4,

wherein the ratio of the dengue-2/1 chimera, to the live, attenuated dengue-2 virus, to the dengue-2/3 chimera, to the dengue-2/4chimera is 5:4:5:5 PFU.

7. The pharmaceutical composition according to any one of claims 1, 3 or 4, or the use according to any one of claims 2 to 4, comprising at least one of

the dengue-2/1 chimera having a concentration from 1.0×10^3 to 5×10^5 PFU;

the live, attenuated dengue-2 virus having a concentration from 1.0×10^3 to 5×10^5 PFU,

the dengue-2/3 chimera having a concentration from 5.0×10^3 to 5×10^5 PFU, and

the dengue-2/4 chimera having a concentration from 1.0×10^4 to 5×10^6 PFU.

8. The pharmaceutical composition according to any one of claims 1, 3 or 4, or the use according to any one of claims 2 to 4, comprising

2.5×10^4 PFU of the dengue-2/1 chimera,

6.3×10^3 PFU of the live, attenuated dengue-2 virus,

3.2×10^4 PFU of a dengue-2/3 chimera, and

4.0×10^5 PFU of a dengue-2/4chimera.

9. The pharmaceutical composition according to any one of claims 1 or 3 to 8, or the use according to any one of claims 2 to 8,

wherein the composition further comprises a stabilizing buffer to reduce degradation of the dengue viruses.

10. The pharmaceutical composition according to claim 9, or the use according to claim 9,

wherein the stabilizing buffer comprises trehalose and albumin and optionally, poloxamer 407.

11. The pharmaceutical composition according to claim 10 or the use according to claim 10,

wherein the poloxamer 407 has a concentration from 0.1 to 3.0 % (w/v),

the trehalose has a concentration from 5.0 to 50% (w/v), and

the albumin has a concentration from 0.01 to 3.0% (w/v).

12. The use according to any one of claims 2 to 11,

wherein the child or young adult is seronegative or naive to dengue virus prior to administration of the pharmaceutical composition.

13. The use according to any one of claims 2 to 11,

wherein the child or young adult is seropositive to dengue virus prior to administration of the pharmaceutical composition.