endemic areas or in preventing further geographic spread of the disease. It is estimated that 3.5 billion people are threatened by infection with dengue virus. In addition, dengue virus is a leading cause of fever in travelers to endemic areas, such as Asia, Central and South America, and the Caribbean. Additionally, DHF/DSS is a leading cause of serious illness and death among children in some Asian and Latin American countries.

[0004] 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 in tropical regions, worldwide. Dengue viruses are transmitted to humans primarily by *Aedes aegypti* 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 leads to an increased risk of severe disease (DHF/DSS) upon secondary infection with a different serotype. The development of an effective vaccine, especially in children and young adults, represents an important approach to the prevention and control of this global disease.

SUMMARY

[0005] Examples disclosed herein concern compositions, methods and uses of chimeric dengue virus constructs. In some examples, a composition can include chimeric dengue virus constructs having an attenuated dengue virus backbone with structural genes from at least one other dengue virus serotype alone or in combination with live, attenuated dengue viruses. Other examples concern at least one live, attenuated virus in combination with one or more chimeric dengue viruses containing structural elements against at least one additional dengue virus serotype. Other examples can include a composition of chimeric dengue viruses having a modified DEN-2 backbone (*e.g.*, PDK-53 as a starting backbone in P1 (passage-1) and modified PDK-53 using passage variability and selection as indicated for P2, P3...P8..P10 etc.) and one or more structural components of DEN-1, DEN-2, DEN-3 or DEN-4. In some examples, an immunogenic composition can be generated where when introduced to a subject, the composition produces an immune response to one or more dengue viruses in the subject. Therefore, constructs contemplated herein can be generated and passaged and each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition

[0006] Examples disclosed herein concern compositions, methods, and uses for inducing an immune response to all four dengue virus serotypes in a child or young adult from about 1 year old to about 45 years of age. Current formulations are not effective against young children and there is an unmet need for dengue vaccines of use against dengue infection for all age groups. In accordance with these examples, compositions and methods disclosed herein are of use against children ages from about 1 to 20 years of age, from about 1 to 15 years of age, from about 1 to 11 years of age or from about 2 to about 9 years of age or from about 1 to about 5 years of age.

[0006a] In one aspect, the present disclosure refers to 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.

[0006b] In another aspect, the present disclosure refers to the 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.

[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.

[0009] In certain examples, an immunogenic composition of the present disclosure can include chimeric dengue viruses against one or more of DEN-1, DEN-2, DEN-3 and/or DEN-4, alone or in combination with a live, attenuated dengue virus composition.

[00010] In other examples, a construct can include a construct having adaptive mutations in the structural or non-structural regions of the virus that increase growth or production without affecting attenuation or safety of the virus when introduced to a subject. In certain examples, any of the contemplated chimeric dengue virus constructs can include a live, attenuated DEN-2 virus having specific mutations used as a backbone where the live attenuated DEN-2 PDK virus can further include structural proteins of one or more of prM (premembrane) and E (envelope) structural proteins of the other dengue virus serotypes. In addition, a DEN-2 backbone can include additional mutations or reversions of mutations in order to enhance the immune response to a predetermine composition in a subject upon administration (*e.g.*, chimeric Dengue virus 2/1, 2/3 or 2/4).

[00011] In some examples, structural protein genes can include prM and E genes of DEN-1, DEN-2, DEN-3 or DEN-4 on a DEN-2 backbone having one or two reversions to improve immunogenicity. For example, a dengue construct, in certain examples can include those constructs termed TDV-1-A, TDV-2-F, TDV-3-F, and TDV-4-F (see Example section) where the DEN-2 backbone has one or more reversions to wild-type DEN-2 (*e.g.*, in the non-coding region (NCR) or a non-structural region (NS1 etc.) or additional mutations not found in the P1 or other previous passaged virus) from the DEN-2 live, attenuated virus previously demonstrated to be safe and effective to induce an immune response. The DEN-2 live, attenuated virus of the instant application is an improved version of the originally used DEN-2 live, attenuated virus. A chimeric construct of the some examples disclosed herein can include a modified attenuated DEN-2 PDK-53 backbone, having one or more structural proteins of the second dengue virus serotype wherein the structural proteins can include additional mutations to increase an immunogenic response to the chimeric construct. In some examples, certain mutations acquired by attenuated DEN-2 PDK-53 can be reverted back to a control or another amino acid to produce chimeric constructs different from the P1 construct which can result in increased immunogenicity, increased growth, increased plaque size without affecting vaccine safety and attenuation and may affect growth and/or replication of live attenuated virus.

[00012] In other examples, a live, attenuated DEN-2 genome can be used to generate chimeric constructs of dengue virus serotype 1 (DEN-1), dengue virus serotype 3 (DEN-3), and dengue virus serotype 4 (DEN-4) where one or more structural protein genes of the DEN-2 viral genome can be replaced by one or more structural protein genes of DEN-1, DEN-3 or DEN-4, respectively. In some examples, a structural protein can include C, prM or E protein of a third dengue virus. In certain examples, structural protein genes can include the prM and E genes of DEN-1, DEN-3 or DEN-4. In accordance with these examples, these chimeric viruses can express surface antigens of DEN-1, DEN-3 or DEN-4 while retaining the attenuation phenotypes of the parent attenuated DEN-2. Further, chimeric constructs disclosed herein of use for vaccines in children and young adults can include chimeric constructs of DEN-4, DEN-2, DEN-1, and DEN-3 expressing surface antigens of DEN-1, DEN-3 and DEN-4 using attenuated DEN-2 virus as a backbone.

[00013] In certain examples, compositions of the instant disclosure can include a composition that can include a single chimeric dengue virus construct disclosed herein and a pharmaceutically acceptable carrier or excipient. In other examples, compositions disclosed herein can include a two or more, or three or more chimeric dengue virus constructs, and a pharmaceutically acceptable carrier or excipient. In accordance with these examples, a one or more dengue virus chimeric constructs contemplated herein can be combined with one or more, live attenuated dengue viruses. In certain examples, a live, attenuated virus can be a live, attenuated DEN-2 virus wherein reversions to wild-type amino acids in the NCR, NS1 regions or other regions increase the immune response, increase viral growth or other improvement for an improved live, attenuated dengue virus construct of use in formulations disclosed herein.

[00014] In certain examples, live, attenuated dengue viruses can include mutations or substitutions for nucleotide 5'NCR-57-T, NS1-53-Asp, and NS3-250-Val, of the DENV-2, that can be shared by the common PDK-53 virus-specific genetic backgrounds of the four TDV construct viruses. Genetic sequences of three attenuation loci, as well as, the previously established *in vitro* and *in vivo* attenuation phenotypes of these vaccine candidates have been carefully monitored for the cGMP-manufactured TDV seeds. Disclosed herein are strategies used to generate master virus seeds (MVS) as well as other relevant passages of dengue viruses of use in the manufacture of dengue virus vaccine compositions. These MVS can be used for manufacture of clinical materials and ultimately commercial vaccine supplies.

[00015] In certain examples, immunogenic compositions can include trivalent or tetravalent formulations capable of inducing an immune response to at least three or all four dengue virus serotypes in children and young adults. For example, immunogenic compositions can include Pre-Master Virus Seed, Master Virus Seed (MVS), Working Virus Seed (WVS) and Bulk Virus Seed (BVS) constructs of each of the four dengue virus serotypes. In other examples, immunogenic compositions can include one or more polynucleotides having nucleic acid sequences encoding a modified live, attenuated dengue-2 virus serotype represented by SEQ ID NOs: 9 (Pre-Master), 11 (MVS), 13 (WVS) or 15 (BVS); a dengue-1/dengue-2 chimeric polynucleotide having a nucleic acid sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus serotype and structural proteins from a dengue-1 virus serotype, represented by SEQ ID NOs: 1 (Pre-Master), 3 (MVS), 5 (WVS) or 7 (BVS); a dengue-3/dengue-2 chimeric polynucleotide having a nucleic acid sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus serotype and structural proteins from a dengue-3 virus serotype, represented by SEQ ID NOs: 17 (Pre-Master), 19 (MVS), 21 (WVS) or 23 (BVS); and a dengue-4/dengue-2 chimeric polynucleotide having a nucleic acid sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus serotype and structural proteins from a dengue-4 virus serotype represented, by SEQ ID NOs: 25 (Pre-Master), 27 (MVS), 29 (WVS) or 31 (BVS). Some examples disclose immunogenic compositions capable of eliciting an immune response against one, two, three or all four dengue virus serotypes in a child or young adult.

[00016] In certain examples, immunogenic compositions can include formulations wherein the concentration ratio of the polynucleotide encoding a modified, live attenuated dengue-2 virus serotype in the composition can be at least one half to one log plaque forming unit (PFU) lower than the log PFU of one or more of the dengue virus polynucleotides. In other examples, immunogenic compositions can include formulations wherein the concentration ratio of the polynucleotide encoding a modified, live attenuated dengue-2 virus serotype in the composition is at least one and one half or greater than two or three logs plaque forming unit (PFU) lower than the log PFU of one or more of the polynucleotides

[00017] In certain examples, immunogenic compositions against dengue alone or in combination with other viral vaccines can be mixtures of polypeptides and polynucleotides. In accordance with these examples, immunogenic compositions can include polynucleotides

described herein or other flavivirus polynucleotides with or without various polypeptides encoded by polynucleotides. For example, a polypeptide can include one that corresponds to the modified live, attenuated dengue-2 virus serotype represented by SEQ ID NOs: 10 (Pre-Master), 12 (MVS), 14 (WVS) or 16 (BVS); a polypeptide corresponding to the dengue-1/dengue-2 chimeric polynucleotide represented by SEQ ID NOs: 2 (Pre-Master), 4 (MVS), 6 (WVS) or 8 (BVS); a polypeptide corresponding to the dengue-3/dengue-2 chimeric polynucleotide represented by SEQ ID NOs: 18 (Pre-Master), 20 (MVS), 22 (WVS) or 24 (BVS); and a polypeptide corresponding to the dengue-4/dengue-2 chimeric polynucleotide represented by SEQ ID NOs: 26 (Pre-Master), 28 (MVS), 30 (WVS) or 32 (BVS).

[00018] In certain examples, immunogenic compositions disclosed herein can include polypeptides and/or polynucleotides at various concentration ratios, including a concentration ratio of a dengue-1/dengue-2 chimera, to a modified live, attenuated dengue-2 virus serotype, to a dengue-3/dengue-2 chimera, to a dengue-4/dengue-2 chimera of 4:4:5:5 log plaque forming units (PFU). In other examples, concentration ratios of a dengue-1/dengue-2 chimera, to a modified live, attenuated dengue-2 virus serotype, to a dengue-3/dengue-2 chimera, to a dengue-4/dengue-2 chimera can be about 5:4:5:5 log PFU. Other ratios contemplated herein can include compositions where live, attenuated dengue-2 and/or dengue-1 chimeric viruses are reduced compared to at least one of dengue-3 or dengue-4 chimeras and/or dengue-1. In yet other examples, tetravalent formulation ratios can be about 5:3:5:5, 3:3:5:5, or any ratio having in certain examples lower live, attenuated dengue-2 or reduced representation or both live, attenuated dengue-2 and dengue-1/dengue-2 chimera in the formulation.

[00019] In other examples, compositions disclosed herein can be a pharmaceutical composition that includes one or more immunogenic composition disclosed herein and a pharmaceutically acceptable excipient. In certain examples, pharmaceutical compositions can include immunogenic compositions made up of live, attenuated dengue viruses and/or dengue chimeras having both polynucleotides and polypeptides directed against one or more dengue serotypes.

[00020] Other examples can include kits that having at least on immunogenic composition of the present disclosure and at least one container of use against dengue virus infection in children and young adults.

[00021] Certain examples include methods for immunizing children and young adults against at least one to all four dengue virus serotypes. Methods can include administering one or more doses of pharmaceutical compositions disclosed herein to a subject. In accordance with these examples, a ratio having these characterizations can include live, attenuated viruses and/or chimeras that induce an immune response to all four dengue virus serotypes in the subject.

[00022] In certain examples, methods can include administering an immunogenic composition to a child or young adult that includes one or more polypeptides encoded by the polynucleotides disclosed herein in addition to polynucleotides. Methods can include polypeptides that include one or more of a polypeptide represented by SEQ ID NOs: 10 (Pre-Master), 12 (MVS), 14 (WVS) or 16 (BVS); a polypeptide represented by SEQ ID NOs: 2 (Pre-Master), 4 (MVS), 6 (WVS) or 8 (BVS); a polypeptide represented by SEQ ID NOs: 18 (Pre-Master), 20 (MVS), 22 (WVS) or 24 (BVS); and a polypeptide represented by SEQ ID NOs: 26 (Pre-Master), 28 (MVS), 30 (WVS) or 32 (BVS). In certain examples, methods can include administering immunogenic compositions having all four dengue virus serotypes represented in the immunogenic compositions disclosed herein to subjects ranging from about 1 year to about 20 years of age and eliciting immune responses to all four dengue virus serotypes (tetravalent formulations) in the subject. In other examples, methods can include administering immunogenic compositions disclosed herein to subjects ranging from about 1 year to about 45 years of age.

[00023] In certain examples, methods can include administering one or more doses of tetravalent formulations of the present disclosure to a subject by any acceptable means, including for example, subcutaneously, intravenously, intradermally, transdermally, orally, via inhalation, intravaginally, topically, intranasally or rectally. In accordance with these examples, formulations disclosed herein can be administered in a single dose or in two or more doses to a subject. In some examples, immunogenic formulations disclosed herein can be administered within about 90 days of each other, within about 60 days of each other, within about 30 days of each other, and within less than about 30 days of each other. In some examples, immunogenic formulations disclosed herein can be administered to a child or young adult on day 0 and again around 3 months from the first administration.

[00024] In certain examples, tetravalent formulations disclosed herein can elicit immune responses against all four dengue virus serotypes in at least 60% of children and young adults having received one or more doses of tetravalent compositions.

Brief Description of the Drawings

[00025] The following drawings form part of the present specification and are included to further demonstrate certain embodiments. Some embodiments may be better understood by reference to one or more of these drawings alone or in combination with the detailed description of specific embodiments presented.

[00026] **Fig. 1** represents an exemplary chart reflecting an exemplary chimeric construct of the instant invention, DEN-2/DEN-4 compared to previously generated constructs and wild type dengue viruses.

[00027] **Fig. 2** represents an exemplary histogram plot comparing various responses using a live, attenuated DEN-2 backbone (with additional mutations) and a second dengue virus serotype as structural components substituted for the dengue-2 structural components (*e.g.*, TDV-1 MVS). This plot illustrates plaque sizes of the TDV MVS. Wild-type Dengue viruses and previously published research-grade vaccine candidate viruses were included for control and comparison. This plot illustrates improved production of the dengue virus constructs compared to control dengue virus chimeric constructs.

[00028] **Fig. 3** represents an exemplary histogram plot that represents temperature sensitivities of TDV MVS (Master Virus Seed, denoted here as DENVax). Wild type dengue viruses and previously published research-grade vaccine candidate viruses were included for comparison with the MVS grade.

[00029] **Fig. 4** represents an exemplary histogram plot that represents viral growth of TDV (also known as DENVax) MVS in C6/36 cells compared to controls. Wild-type dengue viruses and research-grade vaccine candidate viruses were included for comparison with the TDV MVS (denoted here as DENVax).

[00030] **Figs. 5A-5C** represent exemplary plots of neurovirulence in newborn mice. Pooled results of several experiments summarizing the neurovirulence of wt DENV-2 16681 virus in CDC-ICR (n=72) and Taconic-ICR (n=32) newborn mice challenged ic with 10^4 pfu of the virus (A). Neurovirulence of TDV MVS (Master Virus Seed, denoted here as DENVax) tested in Taconic-ICR mice with a dose of 10^4 pfu (B) or 10^3 pfu (C). The numbers of animals

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.

3. The use according to claim 2,

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.

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

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.

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

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.

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

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).

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

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.

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

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

10. The pharmaceutical composition according to any one of claims 1 or 4 to 9, or the use according to any one of claims 2 to 9,

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

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

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

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

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).

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

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

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

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