(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 January 2014 (30.01.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/016362 A1

(51) International Patent Classification:
A61K 39/12 (2006.01) *A61K 39/295* (2006.01)

(21) International Application Number:
PCT/EP2013/065669

(22) International Filing Date:
24 July 2013 (24.07.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
12305908.1 24 July 2012 (24.07.2012) EP
12305911.5 25 July 2012 (25.07.2012) EP

(71) Applicant *(for all designated States except US)*: **SANOFI PASTEUR** [FR/FR]; 2 avenue Pont Pasteur, F-69367 LYON Cedex (07) (FR).

(72) Inventors; and
(71) Applicants *(for US only)*: **YAO, Jiansheng** [CA/CA]; 15, Partridge Lane, Toronto, M1T 3C5 (CA). **GIRERD-CHAMBAZ, Yves** [FR/FR]; 22, chemin de la Pra, F-69510 Messimy (FR). **LEGASTELOIS, Isabelle** [FR/FR]; 392, rue de la Joannas, F-69700 Saint Andeol le Château (FR). **MANTEL, Nathalie** [FR/FR]; 84, rue de la Garenne, F-69005 Lyon (FR). **BARBAN, Véronique** [FR/FR]; 15, rue Sainte Marguerite, F-69110 Sainte-Foy-Les Lyon (FR). **LANG, Jean** [FR/FR]; 118, route de Saint Priest, F-69780 Moins (FR). **GUY, Bruno** [FR/FR]; 2, rue Casimir Perrier, F-69002 Lyon (FR).

(74) Agent: **COMMANDER, Paul, M., B.**; Sanofi Pasteur, Intellectual Property Department, 2 avenue Pont Pasteur, F-69367 Lyon Cédex 07 (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: VACCINE COMPOSITIONS FOR PREVENTION AGAINST DENGUE VIRUS INFECTION

(57) Abstract: The present invention relates to vaccine compositions that are useful in a method of protecting a human subject against dengue disease.

WO 2014/016362 A1

VACCINE COMPOSITIONS FOR PREVENTION AGAINST DENGUE VIRUS INFECTION

FIELD OF THE INVENTION

The present invention relates to vaccine compositions and uses of such compositions in a method of protecting a human subject against dengue disease.

BACKGROUND

Dengue is the second most important infectious tropical disease after malaria with approximately one-half of the world's population living in areas where there is a risk of epidemic transmission. There are estimated to be 50-100 million cases of dengue disease every year resulting in 500,000 patients being hospitalized for dengue hemorrhagic fever (DHF) and resulting in approximately 25,000 deaths.

Dengue disease infections are endemic in more than 100 tropical countries and dengue hemorrhagic fever (DHF) has been documented in 60 of these countries (Gubler, 2002, TRENDS in Microbiology, 10: 100-103).

Dengue disease is caused by four antigenically distinct, but closely related dengue virus serotypes of the flavivirus genus (Gubler et al., 1988, in: Epidemiology of arthropod-borne viral disease. Monath TPM, editor, Boca Raton (FL): CRC Press: 223-60; Kautner et al., 1997, J. of Pediatrics, 131 : 516-524; Rigau-Perez et al., 1998, Lancet, 352: 971-977; Vaughn et al., 1997, J. Infect. Dis., 176: 322-30).

Dengue disease is usually transmitted by injection of the dengue virus during the blood meal of an *Aedes aegypti* mosquito infected by the virus. After an incubation period of 4-10 days, the illness begins abruptly and is followed by three phases: febrile (2 to 7 days), critical (24-48 hours - during which severe complications may occur) and recovery (48-72 hours). During the critical phase, life threatening complications such as hemorrhages, shock and acute organ impairment may occur. A proper management of these unpredictable outcomes can reduce the case fatality rate. Cure of dengue fever is complete after 7 to 10 days, but prolonged asthenia is normal. Reduced leukocyte and platelet numbers are frequently observed.

Dengue haemorrhagic fever (DHF) is a potentially deadly complication of dengue virus infection. DHF is characterized by a high fever and symptoms of dengue disease, but with extreme lethargy and drowsiness. Increased vascular permeability and abnormal homeostasis can lead to a decrease in blood volume, hypotension, and in severe cases, hypovolemic shock and internal bleeding. Two factors appear to play a major role in the occurrence of DHF - rapid viral replication with a high level of viremia (the severity of the disease being associated with the level of viremia; Vaughn et al., 2000, J. Inf. Dis., 181: 2-

9) and a major inflammatory response with the release of high levels of inflammatory mediators (Rothman and Ennis, 1999, Virology, 257: 1-6; Alan L. Rothman. 2011, Nature Reviews Immunology, 11: 532-543)). The mortality rate for DHF can reach 10% without treatment, but is < 1 % in most centres with access to treatment.

Dengue shock syndrome (DSS) is a common progression of DHF and is frequently fatal. DSS results from generalized vasculitis leading to plasma leakage into the extravascular space. DSS is characterized by rapid and poor volume pulse, hypotension, cold extremities, and restlessness.

In Asia, DHF and DSS are observed primarily in children, with approximately 90% of those with DHF being less than 15 years of age (Malavige et al., 2004, Postgrad Med. J., 80: 588–601; Meulen et al., 2000, Trop. Med. Int. Health, 5:325–9). In contrast, outbreaks in the Caribbean and Central America have predominantly affected adults (Malavige et al., 2004, Postgrad Med. J., 80: 588–601).

The four serotypes of dengue virus possess approximately 60-80% sequence homology. Infection with one dengue serotype provides durable homologous immunity but limited heterologous immunity (Sabin, 1952, Am. J. Trop. Med. Hyg., 1: 30-50). Accordingly, an individual that has been infected with one serotype of dengue may subsequently become infected with a different serotype. In the past, it has been considered that a second infection arising from a different dengue virus serotype is theoretically a risk factor for the development of DHF, since the majority of patients that exhibit DHF have been previously exposed to at least one of the other four serotypes of dengue viruses.

To date, there is no specific treatment for dengue disease. Treatment for dengue disease is symptomatic, with bed rest, control of the fever and pain through antipyretics and analgesics, and adequate drinking. The treatment of DHF requires balancing of liquid losses, replacement of coagulation factors and the infusion of heparin.

Since dengue prevention measures, such as mosquito control and personal protection from bites, are limited in efficacy, difficult to enforce and expensive, a safe and efficacious dengue vaccine would be the best mode of prevention. However, there is no licensed vaccine of this type that is currently available.

It is therefore desirable to develop a vaccine composition that demonstrates efficacy when used in a method of protecting a human subject against dengue disease.

SUMMARY OF THE INVENTION

The present invention relates to a dengue virus serotype 2 vaccine composition comprising:

(i) a dengue antigen selected from the group consisting of:
  (a) a live attenuated dengue virus;
  (b) an inactivated dengue virus;
  (c) a live attenuated or inactivated chimeric dengue virus;
  (d) a dengue virus-like particle (VLP); and
  (e) a combination of two or more of (a) to (d);

or

(ii) a nucleic acid construct or viral vector which is able to express in a human cell a dengue antigen which is a dengue VLP;

wherein said dengue antigen comprises a polypeptide having at least 90% identity to SEQ ID NO: 12.

The present invention further relates to a vaccine composition comprising a dengue antigen of serotype 2 selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus; or (d) a combination of two or more of (a) to (c); wherein said dengue antigen comprises a nucleotide sequence encoding a protein comprising a polypeptide or polypeptides as defined in the claims.

A vaccine composition comprising a dengue antigen of serotype 2 selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus; or (d) a combination of two or more of (a) to (c); wherein said dengue antigen comprises a nucleotide sequence having at least 90% sequence identity to a sequence selected from the group consisting of the RNA equivalent of SEQ ID NO: 1, the RNA equivalent of SEQ ID NO: 4, the RNA equivalent of SEQ ID NO: 5, the RNA equivalent of SEQ ID NO: 6, the RNA equivalent of SEQ ID NO: 7 and SEQ ID NO: 25.

The present invention further relates to pharmaceutical formulation comprising a vaccine composition of the present invention and a pharmaceutically acceptable carrier, diluent or excipient.

The present invention further relates to a vaccine composition of the present invention for use in therapy.

The present invention further relates to a vaccine composition of the present invention for use in a method of protecting a human subject against dengue disease caused by a dengue virus of serotype 2.

The present invention further relates to a vaccine composition of the present invention for use in a method of generating neutralising antibodies against a dengue virus of serotype 2.

The present invention further relates to vaccine composition of the present invention which comprises a dengue antigen of serotype 1, a dengue antigen of serotype 2, a dengue antigen of serotype 3 and a dengue antigen of serotype 4 for use in a method of generating neutralising antibodies against the four serotypes of dengue.

The present invention further relates to the use of a vaccine composition of the present invention for the manufacture of a medicament for protecting a human subject against dengue disease caused by a dengue virus of serotype 2.

The present invention further relates to a method of protecting a human subject against dengue disease caused by a dengue virus of serotype 2, wherein said method comprises administering to said subject an effective amount of a composition according to the present invention.

The present invention further relates to a kit comprising a composition according to the present invention and instructions for the use of said composition in a method of protecting a human subject against dengue disease caused by a dengue virus of serotype 2.

The present invention relates to a vaccine composition for use in a method of protecting a human subject against dengue disease, wherein said composition comprises:

(i) a dengue antigen selected from the group consisting of:

(a) a live attenuated dengue virus;

(b) an inactivated dengue virus;

(c) a live attenuated or inactivated chimeric dengue virus;

(d) a dengue virus-like particle (VLP); and

(e) a combination of two or more of (a) to (d);

or

(iii) a nucleic acid construct or viral vector which is able to express in a human cell a dengue antigen which is a dengue VLP.

The present invention further relates to the use of a vaccine composition of the present invention for the manufacture of a medicament for protecting a human subject against dengue disease.

The present invention further relates to a method of protecting a human subject against dengue disease, wherein said method comprises administering to said human subject an effective amount of a composition according to the present invention.

Additionally, the present invention relates to a kit comprising a composition according to the present invention and instructions for the use of said composition in a method of protecting a human subject against dengue disease.

Description of the Figure

Figure 1 illustrates the construction of the YF-VAX cDNA by RT-PCR and cloning

Definitions

The term "Dengue disease", as used herein, refers to the clinical symptoms exhibited by an individual following infection by any one of the four Dengue virus serotypes. Since 1970, clinical dengue has been classified according to World Health Organization guidelines as (i) dengue fever or (ii) dengue hemorrhagic fever (World Health Organization. Dengue hemorrhagic fever: Diagnosis, treatment, prevention and control 2nd Ed. Geneva: WHO, 1997; ISBN 92 4 154500 3). In 2009, the WHO issued new guidelines that classify clinical dengue as (i) dengue with or without warning signs or (ii)

severe dengue. Both classifications are shown in Figures 1 & 2 of Srikiatkachorn et al., Clin. Infect. Dis. (2011) 53(6): 563. According to the earlier classification, dengue fever is characterized by at least two symptoms selected from headache, arthralgia, retro-orbital pain, rash, myalgia, hemorrhagic manifestations, and leucopenia, together with supportive serology or occurrence at the same location and time as other confirmed dengue cases. Progression to Dengue hemorrhagic fever is confirmed when fever, hemorrhagic manifestations, thrombocytopenia and evidence of plasma leakage are all observed. According to the more recent classification, diagnosis of dengue requires the presence of fever and at least two clinical symptoms selected from nausea, vomiting, rash, aches and pains, a positive tourniquet test, or any warning signs selected from abdominal pain and tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy or restlessness, liver enlargement greater than 2 cm or an increase in hematocrit concurrent with a rapid decrease in platelet count. Severe dengue is diagnosed when any of the following events are observed: severe plasma leakage leading to shock or respiratory distress, severe bleeding as evaluated by clinicians or severe organ involvement

The term "Dengue hemorrhagic fever or DHF", as used herein, refers to virologically-confirmed dengue disease wherein fever, hemorrhagic manifestations, thrombocytopenia and evidence of plasma leakage are all observed. DHF, as used herein, may be further defined on the basis of its severity. For instance, DHF may be defined as being of Grade I, Grade II, Grade III or Grade IV (World Health Organization. Dengue hemorrhagic fever: Diagnosis, treatment, prevention and control 2nd Ed. Geneva: WHO, 1997; ISBN 92 4 154500 3). Grade I is defined as fever accompanied by non-specific constitutional symptoms; the only haemorrhagic manifestation is a positive tourniquet test and/or easy bruising. Grade II is defined as spontaneous bleeding in addition to the manifestations of Grade I patients, usually in the form of skin or other haemorrhages. Grade III is defined as circulatory failure manifested by a rapid, weak pulse and narrowing of pulse pressure or hypotension, with the presence of cold clammy skin and restlessness. Grade IV is defined as profound shock with undetectable blood pressure or pulse. As would be understood by a person of skill in the art, in the practice of the present invention, e.g. a method of protecting against DHF, said DHF need not be virologically-confirmed.

The term "virologically-confirmed dengue", as used herein, refers to an acute febrile episode which is confirmed to be induced by a dengue virus, e.g. by reverse transcriptase polymerase chain reaction (RT-PCR) or by a dengue non-structural 1 (NS1) protein enzyme-linked immunosorbent assay (ELISA). In the RT-PCR method, serum samples are tested according to the method of Callahan et al, J. Clin. Microbiol. (2001)

39: 4119. Briefly, RNA is extracted from the serum to discard potential Taq polymerase inhibitors or interfering factors, using a commercial kit. Then an RT-PCR reaction is carried out with serotype specific primers from the dengue NS5 gene sequence. Results are expressed as a concentration of log_{10}GEQ (genome equivalent)/mL, by comparison with standards containing known concentrations of viral genomic serotype-specific nucleic acid sequences integrated into plasmids. In the ELISA method, 50 µL of patient serum, a positive control, a negative control, or a cut-off control are diluted 1:2 in sample diluent and combined with 100 µL of diluted horseradish peroxidase (HRP)-labeled anti-NS1 monoclonal Ab (MAb). The diluted serum and conjugate are added to capture anti-NS1 MAb-coated microwells, and plates are incubated for 90 minutes at 37°C. Capture MAb/NS1/HRP-labeled-MAb complexes are formed when NS1 is present in the serum. Complexes are detected via a colorimetric reaction in positive wells which is induced by adding 160 µL of 3,3',5,5' tetramethylbenzidine (TMB) substrate and incubating for 30 minutes at room temperature in the dark. The reaction is stopped with the addition of 100 µL of stop solution (1N H_2SO4) and the plate is read. A sample ratio is determined for each sample by dividing the average optical density (OD) of the test sample by the average OD of the cut-off control (tested in quadruplicate). Sample ratios of <0.5, 0.5-<1.0, and ≥1 are indicative of negative, equivocal, and positive results, respectively.

The term "severe virologically-confirmed dengue", as used herein, refers to dengue haemorrhagic fever (DHF) as defined by the 1997 WHO classification and further characterized by the following additional list of symptoms: haemorrhage requiring blood transfusion, objective evidence of capillary permeability, signs of circulatory failure or visceral manifestations.

The term "dengue shock syndrome", as used herein, refers to the most severe complications of DHF as defined above. According to the 1997 WHO classification, DSS corresponds to DHF of Grades III and IV.

The term "dengue fever viruses", "dengue viruses" and "DEN" are used interchangeably. They refer to positive single-strand RNA viruses belonging to the Flavivirus genus of the family of flaviviridae. There are four different serotypes of dengue virus (serotypes 1, 2 3 and 4), which possess approximately 60-80% sequence homology. The organization of the genome comprises the following elements: a 5' non-coding region (NCR), a region encoding structural proteins (capsid (C), pre-membrane (prM) and envelope (E)) and a region encoding non-structural proteins (NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5) and a 3' NCR. The dengue viral genome encodes an uninterrupted coding region which is translated into a single polyprotein which undergoes post-translational processing. The sub-sequences included in the prM-E sequences may be

numbered in various ways: (i) the total prM-E protein sequence is numbered from position 1 to position 661, with the preM protein sequence designated as position 1 to position 90/91, the M protein sequence designated as position 91/92 to position 166 and the E protein sequence designated as position 167 to position 661; (ii) the prM and M protein sequences are numbered together, i.e. from position 1 to position 166 of the total sequence and E is numbered separately from position 1 to position 495; (iii) the prM, M and E sequences are numbered separately, i.e. prM is numbered from position 1 to 90/91, M is numbered from 1 to 75/76 and E from position 1 to position 495. In the present disclosure the E protein is always numbered from position 1 to position 495. For example, a residue designated herein as E-154 refers to position 154 of the E protein.

In the context of the present invention, "vaccinal dengue virus" refers to a virus which is capable of inducing neutralizing antibodies against the dengue virus serotype from which the vaccinal dengue virus is derived, by the administration of such vaccinal dengue virus to an immunocompetent subject. Examples of vaccinal dengue viruses which may be used in a method of the present invention include inactivated dengue viruses, live attenuated dengue viruses and live attenuated or inactivated chimeric dengue viruses. Serotypes of vaccinal dengue viruses for use in the present invention include serotypes 1, 2, 3, and 4. Preferably a vaccinal dengue virus for use in the present invention is a live attenuated chimeric dengue virus.

The expression "inactivated virus", as used herein, refers to a virus that is incapable of replication to any significant degree in cells permissive for replication of the corresponding wild type virus. Viruses may be inactivated by a number of means well known to those skilled in the art. Examples of methods for inactivating a virus include chemical treatments, or radiation treatments (including heat or electromagnetic radiation typically in the forms of X-ray or ultraviolet radiation).

The term "inactivated dengue virus", as used herein refers to an inactivated wild-type virus containing all the dengue structural proteins (env, premembrane/membrane and capsid proteins) and inactivated viral RNA. An inactivated dengue virus may also refer to an inactivated chimeric dengue virus. Inactivated dengue viruses are for instance described in United States Patent No. 6,254,873.

The term "live attenuated virus or LAV", as used herein, refers to a virus which is not able to induce a disease state characterised by the same sets of symptoms associated with the corresponding wild-type virus. Examples of live attenuated viruses are well known in the art. A live attenuated virus may be prepared from a wild-type virus, for example, by recombinant DNA technology, site directed mutagenesis, genetic

manipulation, serial passages on replication-competent cells, chemical mutagenesis treatment or electromagnetic radiation.

The term "live attenuated dengue virus", as used herein, refers to a live dengue virus derived from a virulent wild-type dengue virus by genetic modification resulting in attenuation of virulence and an inability to induce a disease state characterised by the same sets of symptoms associated with the corresponding wild type dengue virus. Examples of live attenuated dengue viruses useful in the practice of the present invention include VDV-1, VDV-2, and the strains described for example in applications WO 02/66621, WO 00/57904, WO 00/57908, WO 00/57909, WO 00/57910, WO 02/0950075 and WO 02/102828. Live attenuated dengue viruses of serotype 1 which may be used in the method of the invention include VDV-1. Live attenuated dengue viruses of serotype 2 which may be used in the method of the invention include VDV-2, and LAV-2.

"VDV" and "Vero dengue vaccine" are used interchangeably herein and designate a live attenuated dengue virus capable of replication in Vero cells and capable of inducing a specific humoral response, including the induction of neutralizing antibodies, in a human.

The DEN-1 16007/PDK13 strain, also called "LAV1", is derived from wild-type DEN-1 (dengue virus serotype 1) 16007 strain which has undergone 11 passages through primary dog kidney (PDK) cells (DEN-1 16007/PDK11). LAV1 has been described in patent application EP1 159968 in the name of Mahidol University and has been filed with the National Microorganisms Cultures Collection (CNCM) under number I-2480. "VDV-1" is a virus derived from LAV1 by subsequent adaptation to Vero cells; in this regard, the RNA from LAV1 has been extracted and purified before being transfected into Vero cells. The VDV-1 strain has subsequently been obtained by plate purification and amplification in Vero cells. The VDV-1 strain has 14 additional mutations in comparison with the DEN-1 16007/PDK13 strain (13 passes through PDK cells). A process for preparing and characterizing the VDV-1 strain has been described in international patent application filed under number WO06/134433 in the names of Sanofi-Pasteur and the Center for Disease Control and Prevention.

The DEN-2 16681/PDK53 strain, also known as "LAV2", has been obtained from wild-type strain DEN-2 (dengue virus serotype 2) 16681 which has undergone 50 passes through PDK cells (DEN-2 16681/PDK50). LAV2 has been described in in patent application EP1159968 in the name of Mahidol University and has been filed with the National Microorganisms Cultures Collection (CNCM) under number 1-2481. "VDV-2" is a strain derived from LAV2 by subsequent adaptation to Vero cells; in this regard, the RNA from LAV2 has been extracted and purified before being transfected in Vero cells. The

VDV-2 strain has subsequently been obtained by plate purification and amplification in Vero cells. The VDV-2 strain has 10 additional mutations in comparison with the DEN-2 16681/PDK53 strain (53 passes through PDK cells), including 4 silent mutations. A process for preparing and characterizing the VDV-2 strain has been described in the international patent application filed under number WO06/134443 in the names of Sanofi-Pasteur and the Center for Disease Control and Prevention. The complete nucleic acid sequence of the VDV-2 strain is as shown in SEQ ID NO: 24. The sequence of the E protein of the VDV-2 strain is as shown in SEQ ID NO: 26 and the sequence of the M protein of the VDV-2 strain is as shown in the SEQ ID NO: 27.

The VDV 1 and 2 strains are prepared by amplification in Vero cells. The viruses produced are harvested and clarified from cell debris by filtration. The DNA is digested by treatment with enzymes. Impurities are eliminated by ultrafiltration. Infectious titers may be increased by a concentration method. After adding a stabilizer, the strains are stored in lyophilized or frozen form before use and then reconstituted when needed.

In the context of the invention, “dengue chimera or chimeric dengue virus” means a recipient flavivirus in which the genetic backbone has been modified by exchanging the sequences encoding the prM and E proteins of the recipient flavivirus by the corresponding sequences of a dengue virus. Typically, the recipient flavivirus may be attenuated. The recipient flavivirus may be a yellow fever (YF) virus such as the attenuated YF 17D, YF 17DD and YF 17D204 (YF-VAX®) viruses; in that case, such chimeras are referred to as YF/dengue chimeras. The recipient flavivirus may also be a dengue virus and in that case, it is referred to as dengue/dengue chimera, the dengue virus serotype characteristic of the prM and E proteins being identical or different from the recipient dengue virus serotype characteristic of the genetic backbone. When the serotypes are identical, the recipient dengue virus and the dengue virus from which the prM and E protein encoding sequences originate, are two different virus strains of the same serotype. For use in the present invention, chimeric dengue viruses are typically YF/dengue chimeras. Chimeric dengue viruses are preferably inactivated or live attenuated chimeric dengue viruses. Advantageously, the recipient flavivirus of a live attenuated chimeric dengue virus of the present invention is YF 17D or YF 17D204 (YF-VAX®). According to one embodiment dengue chimera is an inactivated virus. According to an alternative embodiment the dengue chimera is a live attenuated virus. Dengue Chimera that can be used in a vaccine composition of the present invention include Chimerivax™ Dengue Serotype 1 (also known as CYD-1), Chimerivax™ Dengue Serotype 2 (also known as CYD-2), Chimerivax™ Dengue Serotype 3 (also known as CYD-3) and Chimerivax™ Dengue Serotype 4 (also known as CYD-4).

Examples of chimeric dengue viruses useful in the practice of the present invention include the dengue/YF chimeric viruses described in patent application WO 98/37911 and dengue/dengue fever chimeras such as those described in patent applications WO 96/40933 and WO 01/60847.

In one embodiment, the chimeric YF/dengue virus comprises the genomic backbone of the attenuated yellow fever virus strain YF17D (Theiler M. and Smith H.H., 1937, J.Exp.Med., 65. 767-786), e.g. viruses YF17D/DEN-1, YF17D/DEN-2, YF17D/DEN-3 and YF17D/DEN-4. Examples of YF17D strains which may be used include YF17D204 (YF-VAX(R), Sanofi-Pasteur, Swiftwater, PA, USA; Stamaril(R), Sanofi-Pasteur, Marcy l'Etoile, France; ARILVAX(TM), Chiron, Speke, Liverpool, UK; FLAVIMUN(R), Berna Biotech, Bern, Switzerland; YF17D-204 France (X15067, X15062); YF17D-204,234 US (Rice et al., 1985, Science, 229: 726-733), or the related strains YF17DD (Genbank access number U17066), YF17D-213 (Genbank access number U17067) and the strains YF17DD described by Galler et al. (1998, Vaccines, 16(9/10): 1024-1028). In another embodiment, the chimeric YF/dengue virus comprises the genomic backbone of the attenuated yellow fever virus strain YF17D204 (YF-VAX®).

One example of a chimeric dengue virus particularly suitable for use in the practice of the present invention is a "Chimerivax dengue virus". As used herein, a "Chimerivax dengue virus", is a live attenuated chimeric YF/dengue virus which comprises the genomic backbone of a YF17D or YF17D204 (YF-VAX®) virus in which the nucleic acid sequences encoding the pre-membrane (prM) and envelope (E) proteins have been replaced by nucleic acid sequences encoding the corresponding structural proteins of a dengue virus. A preferred chimeric dengue virus for use in the present invention is a live attenuated chimeric YF/dengue virus which comprises the genomic backbone of a YF17D virus in which the nucleic acid sequences encoding the pre-membrane (prM) and envelope (E) proteins have been replaced by nucleic acid sequences encoding the corresponding structural proteins of a dengue virus. A preferred chimeric dengue virus for use in the present invention is a live attenuated chimeric YF/dengue virus which comprises the genomic backbone of a YF17D204 (YF-VAX®) virus in which the nucleic acid sequences encoding the pre-membrane (prM) and envelope (E) proteins have been replaced by nucleic acid sequences encoding the corresponding structural proteins of a dengue virus. Construction of such Chimerivax viruses may be achieved in accordance with, or in substantial accordance with, the teaching of Chambers, et al. (1999, J. Virology 73(4):3095-3101). The particular Chimerivax (CYD) dengue viruses described in the examples have been generated by using prM and E sequences from strains DEN 1 PU0359 (TYP1 140), DEN2 PUO218, DEN3 PaH881/88 and DEN 4 1228 (TVP 980) and

the genomic backbone of YF17D virus. Those particular Chimerivax strains are referred to herein (see the present examples) as “CYD-1”, “CYD-2”, “CYD-3” and “CYD-4” respectively. The preparation of these particular CYD-1, CYD-2, CYD-3 and CYD-4 strains has been described in detail in international patent applications WO 98/37911, WO 03/101397, WO 07/021672, WO 08/007021, WO 08/047023 and WO 08/065315, to which reference may be made for a precise description of the processes for their preparation. Alternatively, other dengue fever virus strains may be used as a source of nucleic acids to facilitate construction of chimeric viruses useful in the practice of the present invention, for example in the construction of other Chimerivax dengue serotype 1 (CYD-1), Chimerivax dengue serotype 2 (CYD-2), Chimerivax dengue serotype 3 (CYD-3) and Chimerivax dengue serotype 4 (CYD-4) strains. Advantageously, a vaccine composition of the present invention, e.g. a chimeric dengue virus, of serotype 2 may comprise prM-E sequences having at least 90%, at least 95%, at least 98% or at least 99% identity to the prM-E sequences from the serotype 2 strains LAV-2, BID-V585, PR/DB023 or MD1280 as described in the examples or may comprise prM-E sequences having at least 90%, at least 95%, at least 98% or at least 99% identity to the prM-E sequence shown in SEQ ID NO: 2. Advantageously, a vaccine composition, e.g. a chimeric dengue virus, of serotype 2 for use in the method of the present invention may comprise prM-E sequences from the serotype 2 strains LAV-2, BID-V585, PR/DB023 or MD1280 or the prM-E sequence from SEQ ID NO: 2 as described in the examples. When the recipient genomic backbone of such chimeric dengue viruses is derived from YF-VAX®, such strains are referred to herein as CYD-LAV, CYD-BID, CYD-PR and CYD-MD. A vaccine composition of the present invention comprising chimeric dengue virus of serotype 2 generated using the prM-E sequences of the serotype 2 strains LAV-2 (SEQ ID NO: 8), BID-V585 (SEQ ID NO: 9), PR/DB023 (SEQ ID NO: 10), MD1280 (SEQ ID NO: 11) or SEQ ID NO: 2, or generated using prM-E sequences having at least 90%, at least 95%, at least 98% or at least 99% identity to the prM-E sequences from the serotype 2 strains LAV-2, BID-V585, PR/DB023, MD1280 or the prM-E sequence from SEQ ID NO: 2 may advantageously be used in combination with CYD-1, CYD-3 and CYD-4 in a vaccine composition according to the present invention. Examples of chimeric dengue virus of serotype 2 generated using the prM-E sequences of the serotype 2 strains LAV-2 (SEQ ID NO: 8), PR/DB023 (SEQ ID NO: 10) and MD1280 (SEQ ID NO: 11) include CYD-LAV, CYD-PR and CYD-MD respectively.

An alternative embodiment of chimeric dengue virus usable in the method of protection of the invention is a recipient flavivirus in which the genetic backbone has been modified by exchanging (i) the sequence encoding the E protein of the recipient flavivirus

by the corresponding sequence of a dengue virus and (ii) the sequence encoding the prM protein of the recipient flavivirus by the corresponding sequence of a non-dengue flavivirus, e.g. a JEV virus. Typically, the said chimeric virus may be a live attenuated virus or an inactivated virus. Examples of such chimeric dengue viruses are described in WO2011/138586.

A vaccinal dengue virus of serotype 1 for use in a vaccine composition of the present invention may, for example, be the strain VDV1, CYD-1 or a YF17D/DEN-1 chimeric virus comprising the prM and E amino acid sequences of the DEN-1 16007/PDK13 strain. A vaccinal dengue virus of serotype 2 for use in the method of the present invention may, for example, be the strain VDV2, CYD-2, a YF17D/DEN-2 chimeric virus comprising the prM and E amino acid sequences of the DEN-2 16681/PDK53 strain, a chimeric virus comprising the prM and E amino acid sequences of the DEN-2 strains LAV-2, BID-V585, PR/DB023 or MD1280 or a chimeric virus comprising prM-E sequences having at least 90%, at least 95%, at least 98% or at least 99% identity to the prM-E sequences from the serotype 2 strains LAV-2, BID-V585, PR/DB023 or MD1280 or at least 90%, at least 95%, at least 98% or at least 99% identity to the prM-E sequence in SEQ ID NO: 2. A vaccinal dengue virus of serotype 3 for use in the method of present invention may, for example, be CYD-3 or an alternative YF17D/DEN-3 chimeric virus. An example of a vaccinal dengue virus of serotype 4 is CYD-4 or an alternative YF17D/DEN-4 chimeric virus.

A composition of the present invention comprises at least one dengue antigen. Typically a composition of the present invention comprises a dengue antigen, e.g. a vaccinal dengue virus, of each of serotypes 1, 2, 3 and 4. Dengue antigens, e.g. vaccinal dengue viruses, of the present invention of each serotype may be as described herein. For instance, a composition of the present invention may advantageously comprise any one of the following combinations of dengue antigens: i) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-LAV, a chimeric dengue virus comprising the prM and E amino acid sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; ii) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-BID, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; (iii) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-PR, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; (iv) a dengue antigen comprising the prM and E sequences of CYD-1, a

dengue antigen comprising the prM and E sequences of CYD-MD, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4;. For instance, a composition of the present invention may also advantageously comprise any one of the following combinations of dengue antigens: i) CYD-1, CYD-LAV, CYD-3 and CYD-4; ii) CYD-1, CYD-BID, CYD-3 and CYD-4; (iii) CYD-1, CYD-PR, CYD-3 and CYD-4 or (iv) CYD-1, CYD-MD, CYD-3 and CYD-4. A composition of the present invention may also advantageously comprise the following combination of dengue antigens: i) a dengue antigen comprising the prM and E sequences of CYD-1, VDV2, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4. For instance, a composition of the present invention may advantageously comprise CYD-1, VDV-2, CYD-3 and CYD-4. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises a sequence having at least 90% identity to the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2. For example, said sequence may be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the prm-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2.

The term "virus-like particles or VLPs", as used herein, refers to virus particles that do not contain replicative genetic material but present at their surface a dengue E protein in a repetitive ordered array similar to the virion structure. Typically, dengue VLPs also contain dengue prM and/or M, and E proteins. VLPs may be produced *in vitro* (Zhang et al, J. Virol. (2011) 30 (8):333). VLPs may also be produced *in vivo*. To that end, nucleic acid constructs (e.g. DNA or RNA constructs) encoding prM and E dengue proteins may be introduced into a cell of a subject, e.g. a human subject, via methods known in the art, e.g. via use of a viral vector. Any viral vector may be used provided it is able to contain and express both prM and E dengue virus sequences. Non-limiting examples of viral vectors that may be used in the method of the present invention include the poxviruses (e.g. the attenuated pox Ankara virus) and the measles virus. For use in the present invention, a particular category of viral vector expressing VLPs *in vivo* includes replication-deficient pseudoinfectious (PIV) viruses, e.g. according to the Replivax™ technology. (Rumyantsev AA, et al. Vaccine. 2011 Jul 18;29(32):5184-94).

The term "replication-defective pseudo-infectious virus", as used herein, refers to a virion particle that is replication-defective *in vivo,* owing to the absence in their genome of an essential sequence of the replicative cycle, for example the sequence encoding a capsid protein. However, the virion particles can propagate in a culture of helper cells that provide for the essential sequence(s) *in trans*. Replication-deficient pseudoinfectious viruses for use in the present invention include any virus according to the above definition which is capable of expressing the prM and E proteins of a dengue virus of any serotype. Examples include replication defective flavivirus / dengue chimeras such as replication defective West Nile virus / dengue, Japanese Encephalitis virus / dengue and YF / dengue chimeras.

The ability of a vaccine composition of the present invention to provoke an immune response in a subject (i.e. induce the production of neutralizing antibodies) can be assessed, for example, by measuring the neutralizing antibody titre raised against the dengue virus serotype(s) comprised within the composition. The neutralizing antibody titre may be measured by the Plaque Reduction Neutralization Test ($PRNT_{50}$) test. Briefly, neutralizing antibody titre is measured in sera collected from vaccinated subjects at least 28 days following administration of a vaccine composition of the present invention. Serial, two-fold dilutions of sera (previously heat-inactivated) are mixed with a constant challenge-dose of each dengue virus of serotype 1, 2, 3 or 4 as appropriate (expressed as PFU/mL). The mixtures are inoculated into wells of a microplate with confluent Vero cell monolayers. After adsorption, cell monolayers are incubated for a few days. The presence of dengue virus infected cells is indicated by the formation of infected foci and a reduction in virus infectivity due to the presence of neutralising antibodies in the serum samples can thus be detected. The reported value (end point neutralization titre) represents the highest dilution of serum at which ≥ 50 % of dengue challenge virus (in foci counts) is neutralized when compared to the mean viral focus count in the negative control wells (which represents the 100% virus load). The end point neutralization titres are presented as continuous values. The lower limit of quantification (LLOQ) of the assay is 10 (1/dil). It is commonly considered that seroconversion occurs when the titer is superior or equal to 10 (1/dil). As PRNT tests may slightly vary from a laboratory to another the LLOQ may also slightly vary. Accordingly, in a general manner, it is considered that seroconversion occurs when the titre is superior or equal to the LLOQ of the test. Neutralising antibody titres were considered in the following references, but the authors did not establish a correlate of protection (Guirakhoo et al, J. Virol. (2004) 78 (9): 4761; Libraty et al, PLoS Medicine (2009) 6 (10); Gunther et al, Vaccine (2011) 29: 3895) and Endy et al, J. Infect. Dis. (2004), 189(6): 990-1000).

The term "$CCID_{50}$" refers to the quantity of virus (e.g. vaccinal virus) infecting 50% of the cell culture. The $CCID_{50}$ assay is a limit dilution assay with statistical titer calculation *(Morrison D et al J Infect Dis. 2010; 201(3):370-7))*.

The term "human subject" is intended to mean males and females of various ages. Preferably a human subject according to the present invention is less than 18 years of age or less than 12 years of age. For example, a human subject according to the present invention may be 0-17 years of age, 0-11 years of age, 4-17 years of age, 4-11 years of age, 4-6 years of age, 6-8 years of age, 8-10 years of age, 2-8 years of age, 2-11 years of age, 2-14 years of age, 9-16 years of age, 12-17 years of age or 18-45 years of age. More preferably, a human subject according to the present invention is 4-11 years of age, 2-14 years of age or 9-16 years of age. A human subject according to the present invention may be at least 9 months old or less than 9 months old. For instance a human subject according to the present invention may be 9 months to 16 years of age, 9 months to 14 years of age, 9 months to 11 years of age or 9 months to 8 years of age. A human subject according to the present invention may be at least 9 months old, with no history of severe allergy to any component of the vaccine composition as defined herein, no congenital or acquired immune deficiency, no symptomatic HIV infection and said subject should not be pregnant or breast feeding.

As used herein, the expression "flavivirus-naïve subject" refers to a subject who has not been infected by a flavivirus nor previously immunized with a flavivirus vaccine, i.e. a serum sample taken from said subject will produce a negative result in a flavivirus ELISA or PRNT assay.

As used herein, the expression "dengue-naïve subject" refers to a subject who has not been infected by a dengue virus nor previously immunized with a dengue vaccine, i.e. a serum sample taken from said subject will produce a negative result in a dengue ELISA or PRNT assay.

As used herein, the expression "flavivirus-immune subject" refers to a subject who has been infected or immunized by a flavivirus before administration of the vaccine composition of the invention, i.e. a serum sample taken from said subject will produce a positive result in a flavivirus ELISA or PRNT assay.

As used herein, the expression "dengue-immune subject" refers to a subject who has been infected by a dengue virus or immunized by a dengue vaccine before administration of the vaccine composition of the present invention, i.e. a serum sample taken from said subject will produce a positive result in a dengue ELISA or PRNT assay.

In accordance with the present invention, a "method of protecting", as used herein, results in a reduction in the severity or in the likelihood of developing dengue disease in a

human subject exposed to a dengue virus. Advantageously, said reduction is statistically significant. For example, a method of protecting, according to the present invention, may result in a reduction in at least one symptom of dengue disease as defined herein or a reduction in a combination of any two or more of those symptoms. The protection may result in any one or more of the following:

(i) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, symptomatic virologically-confirmed dengue disease caused by dengue virus of any serotype;

(ii) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, symptomatic virologically-confirmed dengue disease caused by dengue virus of any one of serotypes 1, 3 or 4;

(iii) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, symptomatic dengue disease caused by dengue virus of any serotype;

(iv) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, symptomatic dengue disease caused by dengue virus of any one of serotypes 1, 3 or 4;

(v) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, severe virologically-confirmed dengue caused by dengue virus of any serotype;

(vi) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, severe dengue disease caused by dengue virus of any serotype;

(vii) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, dengue hemorrhagic fever cases of Grades I to IV caused by dengue virus of any serotype;

(viii) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, DHF cases of Grade I caused by dengue virus of any serotype;

(ix) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, DHF cases of Grade II caused by dengue virus of any serotype;

(x) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, DHF cases of Grade III caused by dengue virus of any serotype;

(xi) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, DHF cases of Grade IV caused by dengue virus of any serotype;

(xii) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, fever or a reduction in the mean duration and/or intensity of fever;

(xiii) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, plasma leakage as defined by a change in haematocrit or a reduction in the mean value for plasma leakage as defined by a change in haematocrit;

(xiv) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, thrombocytopenia or a reduction in the mean value for thrombocytopenia;

(xv) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, increases in the level of liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST);

(xvi) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, hospitalization due to virologically-confirmed dengue disease caused by dengue virus of any serotype;

(xvii) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, hospitalization due to dengue disease caused by dengue virus of any serotype;

(xviii) a statistically significant reduction in the length of hospital stay due to virologically-confirmed dengue disease.

(xix) a statistically significant reduction in the length of hospital stay due to dengue disease.

The duration and intensity of fever are monitored and recorded according to standard hospital procedures. In a human subject, a fever (i.e. a febrile episode) is defined as the observance of two temperature readings of at least 37.5°C measured twice over an interval of at least 4 hours. Measurement of haematocrit, thrombocytopenia and hepatic enzyme levels are standard tests well-known to the person of skill in the art, for example as described in the pharmacopea.

Protection against dengue disease, for example as defined in points (i) to (xix) above, may be demonstrated in respect of dengue disease caused by a particular dengue virus serotype. For example, protection against dengue disease, as defined herein, may be demonstrated in respect of dengue disease caused by a dengue virus of serotype 1, a

dengue virus of serotype 2, a dengue virus of serotype 3 or a dengue virus of serotype 4. Advantageously, protection against dengue disease, as defined herein, may be demonstrated in respect of dengue disease caused by, for example, dengue virus of serotype 1 or serotype 3, dengue virus of serotype 1 or serotype 4, dengue virus of serotype 3 or serotype 4, dengue virus of serotype 1 or serotype 2, dengue virus of serotype 2 or serotype 3, dengue virus of serotype 2 or serotype 4, dengue virus of serotype 1, 2 or 3, dengue virus of serotype 1, 3 or 4, dengue virus of serotype 2, 3 or 4 or dengue virus of serotype 1, 2, 3 or 4.

Protection against dengue disease, as defined herein, may advantageously be demonstrated in particular sub-groups of human subjects. For instance, protection against dengue disease may advantageously be demonstrated in a human subject who is less than 18 years of age or less than 12 years of age. For example, a human subject according to the present invention may be 0-17 years of age, 0-11 years of age, 4-17 years of age, 4-11 years of age, 4-6 years of age, 6-8 years of age, 8-10 years of age, 2-8 years of age, 2-11 years of age, 2-14 years of age, 9-16 years of age, 12-17 years of age or 18-45 years of age. More preferably, a human subject according to the present invention is 4-11 years of age, 2-14 years of age or 9-16 years of age. A human subject according to the present invention may be at least 9 months old or less than 9 months old. For instance a human subject according to the present invention may be 9 months to 16 years of age, 9 months to 14 years of age, 9 months to 11 years of age or 9 months to 8 years of age. A human subject according to the present invention may be at least 9 months old, with no history of severe allergy to any component of the vaccine composition as defined herein, no congenital or acquired immune deficiency, no symptomatic HIV infection and said subject should not be pregnant or breast feeding.

Protection against dengue disease, as defined herein, may advantageously be demonstrated in particular countries, areas or regions of the world. For instance, protection against dengue disease may advantageously be demonstrated in a dengue endemic area. For instance, a dengue endemic area according to the present invention in which protection may be demonstrated may comprise those American countries or parts thereof which fall within the tropics and sub-tropics. A dengue endemic area in which protection may be demonstrated according to the present invention may thus comprise any one or more of the following: Brazil, Venezuela, Colombia, Ecuador, Peru, Bolivia, Paraguay, Panama, Costa Rica, Nicaragua, Honduras, El Salvador, Guatemala, Belize, Mexico, the USA and the islands of the Caribbean. In a particular embodiment, a dengue endemic area of the present invention in which protection may be demonstrated may consist of the following: Brazil, Colombia, Honduras, Mexico and Puerto Rico. A dengue

endemic area in which protection may be demonstrated according to the present invention may also include south Asian and Oceania countries within the tropics and sub-tropics. A dengue endemic area according to the present invention in which protection may be demonstrated may thus consist of any one or more of the following: India, Myanmar (Burma), Thailand, Laos, Vietnam, Cambodia, Indonesia, Malaysia, Singapore, the Philippines, Taiwan, Papua New Guinea and Australia. In a dengue endemic area in which protection may be demonstrated according to the present invention, a particular serotype, strain or genotype of wild type dengue virus may be the dominant circulating strain. For example, a dengue virus of serotype 2 may be characterised as having an Asian I or an Asian/American genotype. Asian/American genotype strains are characterised by at least one of, at least two of, at least three of, at least four of, at least five of or all six of the following residues Arg, Asn, Asp, Thr, Gly and His at positions prM-16, E-83, E-203, E-226, E-228 and E-346 respectively (wherein prM-16 designates position 16 of the prM protein and E-83 etc. designates position 83 of the E protein). Asian I genotype strains are characterised by at least one of, at least two of, at least three of, at least four of, at least five of or all six of the following residues Ile, Lys, Asn, Arg, Glu and Tyr at positions prM-16, E-83, E-203, E-226, E-228 and E-346 respectively (see Table 1 of Hang et al., PLoS NTD, 4(7): e757). A preferred dengue endemic area in which protection may be demonstrated according to the present invention is one in which a dengue virus having an Asian/American genotype is the dominant circulating strain, i.e. at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or 100% of the cases of dengue disease in said dengue endemic area are caused by dengue virus having an Asian/American genotype. A preferred dengue endemic area in which protection may be demonstrated according to the present invention is one in which a dengue virus of any one or more of serotypes 1, 3 or 4 is/are the dominant circulating serotype(s), i.e. at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or 100% of the cases of dengue disease are caused by dengue virus of serotypes 1, 3 or 4.

The term “RNA equivalent” of a given DNA sequence, as used herein, refers to a sequence wherein deoxythymidines have been replaced by uridines. Since the DNA sequences in question constitute the cDNA sequences of the dengue viruses, the equivalent RNA sequences constitute the positive strand RNA of those dengue viruses.

Overview of Several Embodiments

The present inventors have, for the first time, demonstrated the efficacy of a vaccine composition in protecting a human subject against dengue disease.

The present invention relates to a dengue virus serotype 2 vaccine composition comprising:

(i) a dengue antigen selected from the group consisting of:
  (a) a live attenuated dengue virus;
  (b) an inactivated dengue virus;
  (c) a live attenuated or inactivated chimeric dengue virus;
  (d) a dengue virus-like particle (VLP); and
  (e) a combination of two or more of (a) to (d);

or

(ii) a nucleic acid construct or viral vector which is able to express in a human cell a dengue antigen which is a dengue VLP;

wherein said dengue antigen comprises a polypeptide having at least 90% identity to SEQ ID NO: 12.

In preferred embodiments, said polypeptide has at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5% identity or 100% identity with SEQ ID NO: 12.

Preferably, said dengue antigen is selected from the group consisting of a live attenuated dengue virus and a live attenuated or inactivated chimeric dengue virus. Preferably, said dengue antigen is selected from the group consisting of a live attenuated dengue virus and a live attenuated chimeric dengue virus. Preferably, said dengue antigen is a live attenuated chimeric dengue virus.

Preferably said dengue antigen according to the present invention comprises a polypeptide having at least 90% identity to SEQ ID NO: 12, for example at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5% identity or 100% identity to SEQ ID NO: 12 over the full length of SEQ ID NO: 12.

Preferably, said dengue antigen does not comprise the prM-E sequence of CYD-2, as defined herein.

Preferably, said vaccine composition does not comprise CYD-2.

Preferably, said dengue antigen comprises a polypeptide which comprises a valine residue at the position within the polypeptide that corresponds to position 251 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a methionine residue at the position within the polypeptide that corresponds to position 6 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a valine residue at the position within the polypeptide that corresponds to position 129 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises an isoleucine residue at the position within the polypeptide that corresponds to position 129 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises an isoleucine residue at the position within the polypeptide that corresponds to position 141 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises an isoleucine residue at the position within the polypeptide that corresponds to position 164 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises an aspartate residue at the position within the polypeptide that corresponds to position 203 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises an asparagine residue at the position within the polypeptide that corresponds to position 203 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a threonine residue at the position within the polypeptide that corresponds to position 226 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a glycine residue at the position within the polypeptide that corresponds to position 228 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises an isoleucine residue at the position within the polypeptide that corresponds to position 308 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a valine residue at the position within the polypeptide that corresponds to position 308 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a threonine residue at the position within the polypeptide that corresponds to position 478 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a valine residue at the position within the polypeptide that corresponds to position 484 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises an isoleucine residue at the position within the polypeptide that corresponds to position 484 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a isoleucine residue at the position within the polypeptide that corresponds to position 485 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide which comprises a alanine residue at the position within the polypeptide that corresponds to position 491 of SEQ ID NO: 12.

Preferably, said dengue antigen comprises a polypeptide having at least 90% sequence identity to SEQ ID NO: 3.

In preferred embodiments, said polypeptide has at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5% identity or 100% identity with SEQ ID NO: 3.

Preferably, a composition of the present invention comprises a polypeptide having at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5% identity or 100% identity with SEQ ID NO: 3. Preferably a dengue antigen according to the present invention comprises a polypeptide having at least 90% identity to SEQ ID NO: 12, for example at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5% identity or 100% identity to SEQ ID NO: 12 over the full length of SEQ ID NO: 12. Preferably, said dengue antigen comprises a polypeptide which comprises a glycine residue at the position within the polypeptide that corresponds to position 15 of SEQ ID NO: 3.

Preferably, said dengue antigen comprises a polypeptide which comprises a serine residue at the position within the polypeptide that corresponds to position 15 of SEQ ID NO: 3.

Preferably, said dengue antigen comprises a polypeptide which comprises a leucine residue at the position within the polypeptide that corresponds to position 24 of SEQ ID NO: 3.

Preferably, said dengue antigen comprises a polypeptide which comprises an isoleucine residue at the position within the polypeptide that corresponds to position 39 of SEQ ID NO: 3.

Preferably, said dengue antigen comprises a polypeptide which comprises a methionine residue at the position within the polypeptide that corresponds to position 39 of SEQ ID NO: 3.

Preferably, said dengue antigen comprises a polypeptide which comprises a valine residue at the position within the polypeptide that corresponds to position 120 of SEQ ID NO: 3.

Preferably, said dengue antigen comprises a polypeptide which comprises an alanine residue at the position within the polypeptide that corresponds to position 120 of SEQ ID NO: 3.

Preferably, said dengue antigen comprises a polypeptide which comprises a threonine residue at the position within the polypeptide that corresponds to position 125 of SEQ ID NO: 3.

Preferably, the polypeptides as defined herein (as comprised within the dengue antigens as comprised within the vaccine compositions of the present invention) comprise a threonine residue at the position within the polypeptide that corresponds to position 125 of SEQ ID NO: 3 and a valine residue at the position within the polypeptide that corresponds to position 417 of SEQ ID NO: 3.

Preferably, the polypeptides which are encoded by the nucleotide sequences as defined herein (i.e as comprised within the vaccine compositions of the present invention) comprise a leucine residue at the position within the polypeptide that corresponds to position 24 of SEQ ID NO: 3, a threonine residue at the position within the polypeptide that corresponds to position 125 of SEQ ID NO: 3 and a valine residue at the position within the polypeptide that corresponds to position 417 of SEQ ID NO: 3.

Preferably, a polypeptide (as comprised within a dengue antigen as comprised within a vaccine composition of the invention) comprises (i) the sequence as set forth in SEQ ID NO: 13 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 13; (ii) the sequence as set forth in SEQ ID NO: 14 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 14; (iii) the sequence as set forth in SEQ ID NO: 15 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 15; (iv) the sequence as set forth in SEQ ID NO: 16 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 16; (v) the sequence as set forth in SEQ ID NO: 18 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 18; or (vi) the sequence as set forth in SEQ ID NO: 26 or a sequence having

at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 26. Preferably when said sequences comprise an amino acid substitution, said sequences have at least 1 and no more than 4 amino acid substitutions, preferably at least 1 and no more than 3 amino acid substitutions, preferably 1 or 2 amino acid substitutions, preferably 1 amino acid substitution. Preferably at most two, preferably one preferably none of the substitutions are high impact amino acid substitutions (i.e. achieving a score of > 25 in the impact scoring method disclosed in Example 2); preferably at most three, preferably two, preferably one, preferably none of the substitutions are median impact amino acid substitutions (i.e. achieving a score of >10 to 25 in the impact scoring method disclosed in Example 2); preferably at most five, preferably four, preferably three, preferably two, preferably one, preferably none of the substitutions are low impact amino acid substitutions (i.e. achieving a score of >0 to 10 in the impact scoring method disclosed in Example 2); preferably all said substitutions are no impact amino acid substitutions (i.e. achieving a score of 0 in the impact scoring method disclosed in Example 2). Preferably said substitutions do not occur at the positions within said sequences corresponding to positions 226, 228 and 251 of SEQ ID NO: 12. Preferably a dengue antigen comprising said polypeptide leads to a balanced immune response when used in the context of a tetravalent composition. Preferably when a vaccine composition comprising a dengue antigen comprising said polypeptide further comprises a dengue antigen of serotypes 1, 3 and 4 as defined herein, said vaccine composition produces a balanced immune response when administered to a mammal, preferably a human.

Preferably said dengue antigen comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 13; SEQ ID NO: 14, SEQ ID NO: 15 SEQ ID NO: 16; SEQ ID NO: 18 and SEQ ID NO: 26.

Preferably said dengue antigen comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 13 and SEQ ID NO: 16.

Preferably a dengue antigen (as comprised within a vaccine composition of the invention) comprises a polypeptide comprising: (i) the sequence as set forth in SEQ ID NO: 19 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 19; (ii) the sequence as set forth in SEQ ID NO: 20 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 20; (iii) the sequence as set forth in SEQ ID NO: 21 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 21; (iv) the sequence as set forth in SEQ ID NO: 22 or a sequence having at least 1 and

no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 22; (v) the sequence as set forth in SEQ ID NO: 23 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 23 or (vi) the sequence as set forth in SEQ ID NO: 27 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 27. Preferably when said sequences comprise an amino acid substitution, said sequences have at least 1 and no more than 4 amino acid substitutions, preferably at least 1 and no more than 3 amino acid substitutions, preferably 1 or 2 amino acid substitutions, preferably 1 amino acid substitution. Preferably at most two, preferably one preferably none of the substitutions are high impact amino acid substitutions (i.e. achieving a score of > 25 in the impact scoring method disclosed in Example 2); preferably at most three, preferably two, preferably one, preferably none of the substitutions are median impact amino acid substitutions (i.e. achieving a score of >10 to 25 in the impact scoring method disclosed in Example 2); preferably at most five, preferably four, preferably three, preferably two, preferably one, preferably none of the substitutions are low impact amino acid substitutions (i.e. achieving a score of >0 to 10 in the impact scoring method disclosed in Example 2); preferably all said substitutions are no impact amino acid substitutions (i.e. achieving a score of 0 in the impact scoring method disclosed in Example 2). Preferably said substitutions do not occur at the position within said sequences corresponding to position 125 of SEQ ID NO: 3. Preferably a dengue antigen comprising said polypeptide leads to a balanced immune response when used in the context of a tetravalent composition. Preferably when a vaccine composition comprising a dengue antigen comprising said polypeptide further comprises a dengue antigen of serotypes 1, 3 and 4 as defined herein, said vaccine composition produces a balanced immune response when administered to a mammal, preferably a human.

Preferably a dengue antigen (as comprised within a vaccine composition of the invention) comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 19; SEQ ID NO: 20, SEQ ID NO: 21; SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 27.

Preferably a dengue antigen (as comprised within a vaccine composition of the invention) comprises:

i) a polypeptide having the sequence as set forth in SEQ ID NO: 13 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 13; and

a polypeptide having the sequence as set forth in SEQ ID NO: 19 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 19;

ii) a polypeptide having the sequence as set forth in SEQ ID NO: 14 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 14; and
a polypeptide having the sequence as set forth in SEQ ID NO: 20 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 20;

iii) a polypeptide having the sequence as set forth in SEQ ID NO: 15 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 15; and
a polypeptide having the sequence as set forth in SEQ ID NO: 21 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 21;

iv) a polypeptide having the sequence as set forth in SEQ ID NO: 16 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 16; and
a polypeptide having the sequence as set forth in SEQ ID NO: 22 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 22;

v) a polypeptide having the sequence as set forth in SEQ ID NO: 18 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 18; and
a polypeptide having the sequence as set forth in SEQ ID NO: 23 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 23; or

vi) a polypeptide having the sequence as set forth in SEQ ID NO: 26 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 26; and
a polypeptide having the sequence as set forth in SEQ ID NO: 27 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 27.

Preferably when said sequences comprise an amino acid substitution, said sequences have at least 1 and no more than 4 amino acid substitutions, preferably at least 1 and no more than 3 amino acid substitutions, preferably 1 or 2 amino acid

substitutions, preferably 1 amino acid substitution. Preferably at most two, preferably one preferably none of the substitutions are high impact amino acid substitutions (i.e. achieving a score of >25 in the impact scoring method disclosed in Example 2); preferably at most three, preferably two, preferably one, preferably none of the substitutions are median impact amino acid substitutions (i.e. achieving a score of >10 to 25 in the impact scoring method disclosed in Example 2); preferably at most five, preferably four, preferably three, preferably two, preferably one, preferably none of the substitutions are low impact amino acid substitutions (i.e. achieving a score of >0 to 10 in the impact scoring method disclosed in Example 2); preferably all said substitutions are no impact amino acid substitutions (i.e. achieving a score of 0 in the impact scoring method disclosed in Example 2). Preferably said substitutions do not occur at the positions within said sequences corresponding to positions 226, 228 and 251 of SEQ ID NO: 12 and the position corresponding to position 125 of SEQ ID NO: 3. Preferably a dengue antigen comprising said polypeptides leads to a balanced immune response when used in the context of a tetravalent composition. Preferably when a vaccine composition comprising a dengue antigen comprising said polypeptides further comprises a dengue antigen of serotypes 1, 3 and 4 as defined herein, said vaccine composition produces a balanced immune response when administered to a mammal, preferably a human.

Preferably a dengue antigen (as comprised within a vaccine composition of the invention) comprises: i) a polypeptide of SEQ ID NO: 13 and a polypeptide of SEQ ID NO: 19; ii) a polypeptide of SEQ ID NO: 14 and a polypeptide of SEQ ID NO: 20; iii) a polypeptide of SEQ ID NO: 15 and a polypeptide of SEQ ID NO: 21; iv) a polypeptide of SEQ ID NO: 16 and a polypeptide of SEQ ID NO: 22; v) a polypeptide of SEQ ID NO: 18 and a polypeptide of SEQ ID NO: 23 or vi) a polypeptide of SEQ ID NO: 26 and a polypeptide of SEQ ID NO: 27.

Preferably said dengue antigen comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 8; SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11.

Preferably said dengue antigen comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 11.

Preferably a composition of the present invention comprises a dengue antigen selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus; or (d) a combination of two or more of (a) to (c); wherein said dengue antigen comprises a nucleotide sequence encoding a polypeptide comprising a polypeptide as defined herein.

The present invention is also directed to a vaccine composition comprising a dengue antigen of serotype 2 selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus; or (d) a combination of two or more of (a) to (c); wherein said dengue antigen comprises a nucleotide sequence having at least 90% sequence identity to a sequence selected from the group consisting of the RNA equivalent of SEQ ID NO: 1, the RNA equivalent of SEQ ID NO: 4, the RNA equivalent of SEQ ID NO: 5, the RNA equivalent of SEQ ID NO: 6, the RNA equivalent of SEQ ID NO: 7 and SEQ ID NO: 25. References to at least 90% sequence identity to the RNA equivalent of SEQ ID NO: 1, the RNA equivalent of SEQ ID NO: 4, the RNA equivalent of SEQ ID NO: 5, the RNA equivalent of SEQ ID NO: 6, the RNA equivalent of SEQ ID NO: 7 or SEQ ID NO: 25 may preferably be read herein as at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5% or 100% sequence identity. When nucleotide sequences of this embodiment of the invention encode polypeptides comprising one or more amino acid substitutions with respect to the polypeptides encoded by SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 25, preferably at most two, preferably one preferably none of the substitutions are high impact amino acid substitutions (i.e. achieving a score of >25 in the impact scoring method disclosed in Example 2); preferably at most three, preferably two, preferably one, preferably none of the substitutions are median impact amino acid substitutions (i.e. achieving a score of >10 to 25 in the impact scoring method disclosed in Example 2); preferably at most five, preferably four, preferably three, preferably two, preferably one, preferably none of the substitutions are low impact amino acid substitutions (i.e. achieving a score of >0 to 10 in the impact scoring method disclosed in Example 2); preferably all said substitutions are no impact amino acid substitutions (i.e. achieving a score of 0 in the impact scoring method disclosed in Example 2). Preferably said substitutions do not occur at the positions within said polypeptides corresponding to positions 226, 228 and 251 of SEQ ID NO: 12 and the positions within said polypeptides corresponding to positions 24 and 125 of SEQ ID NO: 3. Preferably the vaccine compositions comprising a dengue antigen of serotype 2 of this embodiment of the invention lead to a balanced immune response when used in the context of a tetravalent composition. Preferably a dengue antigen of serotype 2 according to this embodiment of the invention further comprises a dengue antigen of serotype 1, a dengue antigen of serotype 3 and a dengue antigen of serotype 4 as described elsewhere herein. Preferably when a vaccine composition comprising a dengue antigen of serotype 2 according to this embodiment of the invention further comprises a dengue antigen of serotypes 1, 3 and 4

as defined herein, said vaccine composition produces a balanced immune response when administered to a mammal, preferably a human. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 7, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 7, wherein said dengue antigen is not CYD-MD or (ii) a vaccine composition comprising a dengue antigen which is CYD-MD. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 7, wherein said vaccine composition does not comprise prM-E sequences from MD1280 or (ii) a vaccine composition comprising prM-E sequences from MD1280. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 7, wherein said vaccine composition does not comprise a dengue antigen comprising the M and E sequences of CYD-MD or (ii) a vaccine composition comprising a dengue antigen comprising the M and E sequences of CYD-MD. Said vaccine composition is also preferably either (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 7, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequences of MD1280 (SEQ ID NO: 11) or (ii) a vaccine composition comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequences of MD1280 (SEQ ID NO: 11). Said vaccine composition is also preferably either (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 7, wherein said vaccine composition does not comprise a dengue antigen comprising the prM and E sequences of CYD-MD or (ii) a vaccine composition comprising a dengue antigen comprising the prM and E sequences of CYD-MD. Said vaccine composition is also preferably either (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 7, wherein said vaccine composition does not comprise a dengue antigen comprising a polypeptide of SEQ ID NO: 16 and a polypeptide of SEQ ID NO: 22 (or a dengue antigen comprising a nucleotide sequence encoding a protein comprising said polypeptides) or (ii) a vaccine composition comprising a dengue antigen comprising a polypeptide of SEQ ID NO: 16 and a polypeptide of SEQ ID NO: 22 (or a dengue antigen comprising a nucleotide sequence encoding a protein

comprising said polypeptides). Preferably, a vaccine composition of the present invention which comprises a dengue antigen of serotype 2 which comprises a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 7 does not comprise: (i) a chimeric virus comprising the prM and E amino acid sequences of MD1280 or (ii) a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-MD (SEQ ID NO: 11). When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 4, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 4, wherein said dengue antigen is not CYD-LAV or (ii) a vaccine composition comprising a dengue antigen which is CYD-LAV. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 4, wherein said vaccine composition does not comprise prM-E sequences from LAV2 or (ii) a vaccine composition comprising prM-E sequences from LAV2. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 4, wherein said vaccine composition does not comprise a dengue antigen comprising the M and E sequences of CYD-LAV or (ii) a vaccine composition comprising a dengue antigen comprising the M and E sequences of CYD-LAV. Said vaccine composition is also preferably either (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 4, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequences of LAV2 (SEQ ID NO: 8) or (ii) a vaccine composition comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequences of LAV2 (SEQ ID NO: 8). Said vaccine composition is also preferably either (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 4, wherein said vaccine composition does not comprise a dengue antigen comprising the prM and E sequences of CYD-LAV or (ii) a vaccine composition comprising a dengue antigen comprising the prM and E sequences of CYD-LAV. Said vaccine composition is also preferably either (i) a vaccine composition comprising a dengue antigen comprising a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 4, wherein said vaccine composition does not comprise a dengue antigen comprising a polypeptide of SEQ ID NO: 13 and a polypeptide of SEQ ID NO: 19 (or a dengue antigen comprising a nucleotide sequence

encoding a protein comprising said polypeptides) or (ii) a vaccine composition comprising a dengue antigen comprising a polypeptide of SEQ ID NO: 13 and a polypeptide of SEQ ID NO: 19 (or a dengue antigen comprising a nucleotide sequence encoding a protein comprising said polypeptides). Preferably, a vaccine composition of the present invention which comprises a dengue antigen of serotype 2 which comprises a nucleotide sequence having at least 90% identity to the RNA equivalent of SEQ ID NO: 4 does not comprise: (i) a chimeric virus comprising the prM and E amino acid sequences of LAV2 or (ii) a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-LAV (SEQ ID NO: 8).

The present invention is also directed to a vaccine composition comprising a dengue antigen of serotype 2 selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus; or (d) a combination of two or more of (a) to (c); wherein said dengue antigen comprises a nucleotide sequence having at least 1 and no more than 20 nucleotide substitutions with respect to a sequence selected from the group consisting of the RNA equivalent of SEQ ID NO: 1, the RNA equivalent of SEQ ID NO: 4, the RNA equivalent of SEQ ID NO: 5, the RNA equivalent of SEQ ID NO: 6, the RNA equivalent of SEQ ID NO: 7 and SEQ ID NO: 25. When nucleotide sequences of this embodiment of the invention encode polypeptides comprising one or more amino acid substitutions with respect to the polypeptides encoded by SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 25, preferably at most two, preferably one preferably none of the substitutions are high impact amino acid substitutions (i.e. achieving a score of >25 in the impact scoring method disclosed in Example 2); preferably at most three, preferably two, preferably one, preferably none of the substitutions are median impact amino acid substitutions (i.e. achieving a score of >10 to 25 in the impact scoring method disclosed in Example 2); preferably at most five, preferably four, preferably three, preferably two, preferably one, preferably none of the substitutions are low impact amino acid substitutions (i.e. achieving a score of >0 to 10 in the impact scoring method disclosed in Example 2); preferably all said substitutions are no impact amino acid substitutions (i.e. achieving a score of 0 in the impact scoring method disclosed in Example 2). Preferably said substitutions do not occur at the positions within said polypeptides corresponding to positions 226, 228 and 251 of SEQ ID NO: 12 and the positions within said polypeptides corresponding to positions 24 and 125 of SEQ ID NO: 3. Preferably the vaccine compositions comprising a dengue antigen of serotype 2 of this embodiment of the invention lead to a balanced immune response when used in the context of a tetravalent composition. Preferably a dengue antigen of serotype 2 according to this embodiment of the invention further comprises a dengue antigen of serotype 1, a

dengue antigen of serotype 3 and a dengue antigen of serotype 4 as described elsewhere herein. Preferably when a vaccine composition comprising a dengue antigen of serotype 2 according to this embodiment of the invention further comprises a dengue antigen of serotypes 1, 3 and 4 as defined herein, said vaccine composition produces a balanced immune response when administered to a mammal, preferably a human.

When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 as defined herein which comprises a polypeptide having at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5% or 100% identity to SEQ ID NO : 12, wherein said polypeptide comprises an isoleucine residue at the position within the polypeptide that corresponds to position 485 of SEQ ID NO : 12 or an alanine residue at the position within the polypeptide that corresponds to position 491 of SEQ ID NO : 12, said vaccine composition preferably (i) is not CYD-MD; (ii) does not comprise the prM-E sequence from MD1280; (iii) does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequences of MD1280 (SEQ ID NO: 11); (iv) does not comprise a dengue antigen comprising the prM and E sequences of CYD-MD; (v) does not comprise a dengue antigen comprising a polypeptide of SEQ ID NO: 16 and a polypeptide of SEQ ID NO: 22 (or a dengue antigen comprising a nucleotide sequence encoding a protein comprising said polypeptides); (vi) does not comprise a chimeric virus comprising the prM and E amino acid sequences of MD1280; (vii) does not comprise a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-MD (SEQ ID NO: 11) and/or (viii) does not comprise a dengue antigen of serotype 2 which comprises the M and E sequences of CYD-MD. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 as defined herein which comprises a polypeptide having at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5% or 100% identity to SEQ ID NO : 12, wherein said polypeptide comprises a methionine residue at the position within the polypeptide that corresponds to position 6 of SEQ ID NO : 12 or a threonine residue at the position within the polypeptide that corresponds to position 478 of SEQ ID NO : 12, said vaccine composition preferably (i) is not CYD-LAV; (ii) does not comprise the prM-E sequence from LAV2; (iii) does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequences of LAV2 (SEQ ID NO: 8); (iv) does not comprise a dengue antigen comprising the prM and E sequences of CYD-LAV; (v) does not comprise a dengue antigen comprising a polypeptide of SEQ ID NO: 13 and a polypeptide of SEQ ID NO: 19 (or a dengue antigen comprising a nucleotide sequence encoding a protein comprising said polypeptides); (vi) does not comprise a chimeric virus comprising the prM and E amino acid sequences of LAV2 ; (vii) does not comprise a dengue antigen of

serotype 2 which comprises the prM-E sequence of CYD-LAV (SEQ ID NO: 8) and/or (viii) does not comprise a dengue antigen of serotype 2 which comprises the M and E sequences of CYD-LAV.

Preferably said dengue antigen comprises a polypeptide which comprises no more than 1, no more than 2, no more than 3, no more than 4, no more than 5, nor more than 6, no more than 7, no more than 8, no more than 9, no more than 10, no more than 11 or no more than 12 minor amino acid residues, wherein a minor amino acid residue at a given position of a prM-E or E sequence is defined as an amino acid that appears in less than 15% of dengue virus prM-E or E sequences of serotype 2 at that position.

Preferably the dengue disease according to the present invention is virologically-confirmed dengue disease.

Preferably a human subject according to the present invention is less than 18 years of age or less than 12 years of age. For example, a human subject according to the present invention may be 0-17 years of age, 0-11 years of age, 4-17 years of age, 4-11 years of age, 4-6 years of age, 6-8 years of age, 8-10 years of age, 2-8 years of age, 2-11 years of age, 2-14 years of age, 9-16 years of age, 12-17 years of age or 18-45 years of age. More preferably, a human subject according to the present invention is 4-11 years of age, 2-14 years of age or 9-16 years of age. A human subject according to the present invention may be at least 9 months old or less than 9 months old. For instance a human subject according to the present invention may be 9 months to 16 years of age, 9 months to 14 years of age, 9 months to 11 years of age or 9 months to 8 years of age. A human subject according to the present invention may be at least 9 months old, with no history of severe allergy to any component of the vaccine composition as defined herein, no congenital or acquired immune deficiency, no symptomatic HIV infection and said subject should not be pregnant or breast feeding.

A human subject to which a vaccine composition of the present invention is to be administered is preferably a person at risk of infection, for instance a person travelling in an area where dengue fever is present, i.e. a dengue endemic area, or a resident of such an area. Preferably a human subject of the present invention resides in a dengue endemic area. Dengue endemic areas according to the present invention include most of the tropics and sub-tropics, for instance any country identified as an endemic country by the WHO. For instance, a dengue endemic area according to the present invention may comprise those American countries or parts thereof which fall within the tropics and sub-tropics. A dengue endemic area according to the present invention may thus comprise any one or more of the following: Brazil, Venezuela, Colombia, Ecuador, Peru, Bolivia, Paraguay, Panama, Costa Rica, Nicaragua, Honduras, El Salvador, Guatemala, Belize,

Mexico, the USA and the islands of the Caribbean. In a particular embodiment, a dengue endemic area of the present invention may consist of the following: Brazil, Colombia, Honduras, Mexico and Puerto Rico. A dengue endemic area according to the present invention may also include south Asian and Oceania countries within the tropics and sub-tropics. A dengue endemic area according to the present invention may thus consist of any one or more of the following: India, Myanmar (Burma), Thailand, Laos, Vietnam, Cambodia, Indonesia, Malaysia, Singapore, the Philippines, Taiwan, Papua New Guinea and Australia. In a dengue endemic area according to the present invention, a particular serotype, strain or genotype of wild type dengue virus may be the dominant circulating strain. For example, a dengue virus of serotype 2 may be characterised as having an Asian I or an Asian/American genotype. Asian/American genotype strains are characterised by at least one of, at least two of, at least three of, at least four of, at least five of or all six of the following residues Arg, Asn, Asp, Thr, Gly and His at positions prM-16, E-83, E-203, E-226, E-228 and E-346 respectively (wherein prM-16 designates position 16 of the prM protein and E-83 etc. designates position 83 of the E protein). Asian I genotype strains are characterised by at least one of, at least two of, at least three of, at least four of, at least five of or all six of the following residues Ile, Lys, Asn, Arg, Glu and Tyr at positions prM-16, E-83, E-203, E-226, E-228 and E-346 respectively (see Table 1 of Hang et al., PLoS NTD, 4(7): e757). A preferred dengue endemic area according to the present invention is one in which a dengue virus having an Asian/American genotype is the dominant circulating strain, i.e. at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or 100% of the cases of dengue disease in said dengue endemic area are caused by dengue virus having an Asian/American genotype. A preferred dengue endemic area according to the present invention is one in which a dengue virus of any one or more of serotypes 1, 3 or 4 is/are the dominant circulating serotype(s), i.e. at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or 100% of the cases of dengue disease are caused by dengue virus of serotypes 1, 3 or 4.

A vaccine composition of the present invention may be administered to a flavivirus immune subject, for example a dengue-immune subject, or a vaccine composition of the present invention may be administered to a flavivirus-naïve subject. Advantageously, a vaccine composition of the present invention is administered to a flavivirus-immune subject, for example a dengue-immune subject.

Preferably, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the likelihood or severity of DHF. A reduction in the likelihood of DHF (i.e. a reduction in the probability of contracting

DHF) may be measured by comparing the number of cases of DHF in a group of subjects who have received a vaccine composition according to the present invention and the number of cases of DHF in a control group of subjects who have not received a vaccine composition according to the present invention. A reduction in the severity of DHF may be determined by calculating the number of subjects displaying DHF of each of Grades I, II, III or IV in a group of subjects who have received a vaccine composition according to the present invention and comparing those numbers to the equivalent numbers from a control group of subjects who have not received a vaccine composition according to the present invention. For instance, a composition for use in a method according to the present invention preferably reduces the number of cases of Grade I DHF, the number of cases of Grade II DHF, the number of cases of Grade III DHF and/or the number of cases of Grade IV DHF in those subjects receiving the vaccine, when compared to the equivalent number of cases Grade I DHF, Grade II DHF, Grade III DHF and Grade IV DHF occurring in a control group of subjects who have not received a vaccine composition according to the present invention.

Preferably, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the incidence or likelihood of symptomatic virologically-confirmed dengue disease. Advantageously, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the incidence or likelihood of symptomatic virologically-confirmed dengue disease caused by dengue virus of serotypes 1, 3 or 4. Advantageously, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the incidence or likelihood of symptomatic virologically-confirmed dengue disease caused by dengue virus of serotypes 1, 2, 3 or 4. Preferably, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the rate of hospitalization due to virologically-confirmed dengue disease, i.e. reduces the incidence of hospitalized virologically-confirmed dengue disease. For instance, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the rate of hospitalization due to virologically-confirmed dengue disease caused by dengue virus of serotypes 1, 3 or 4, i.e. reduces the incidence of hospitalized virologically-confirmed dengue disease caused by dengue virus of serotypes 1, 3 or 4.

Preferably, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the incidence or likelihood of dengue disease. Advantageously, a composition according to the present invention,

e.g. a composition for use in a method according to the present invention, reduces the incidence or likelihood of dengue disease caused by dengue virus of serotypes 1, 3 or 4. Advantageously, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the incidence or likelihood of dengue disease caused by dengue virus of serotypes 1, 2, 3 or 4. Preferably, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the rate of hospitalization due to dengue disease, i.e. reduces the incidence of hospitalized dengue disease. For instance, a composition according to the present invention, e.g. a composition for use in a method according to the present invention, reduces the rate of hospitalization due to dengue disease caused by dengue virus of serotypes 1, 3 or 4, i.e. reduces the incidence of hospitalized dengue disease caused by dengue virus of serotypes 1, 3 or 4.

A vaccine composition according to the present invention may be administered in multiple doses. Doses of a vaccine composition according to the present invention may be administered in an initial vaccination regimen followed by booster vaccinations. For example, a vaccine composition according to the present invention may be administered in one, two or three doses or more than three doses, e.g. four doses. Preferably, the first dose and the third dose are to be administered approximately twelve months apart. For example, an initial vaccination regimen according to the present invention is administered in three doses, wherein the first and third doses of said vaccination regimen are to be administered approximately twelve months apart. Advantageously, a vaccine composition according to the present invention is to be administered in a first dose, a second dose and a third dose. In such an embodiment, said first dose and said third dose may be administered approximately twelve months apart. For instance, a vaccine composition of the present invention may be administered in a first dose, a second dose and a third dose, wherein said second dose is to be administered about six months after said first dose and wherein said third dose is to be administered about twelve months after said first dose. Alternatively, the three doses may be administered at zero months, at about three to four months (e.g. at about three-and-a-half months) and at about twelve months (i.e. a regimen wherein the second dose of the composition is administered at about three-and-a-half months after the first dose, and wherein the third dose of the composition is administered at about twelve months after the first dose).

A vaccine composition according to the present invention may be administered in two doses. Preferably, the first dose and the second dose are to be administered approximately about six to twelve months after the first dose months apart. Preferably, the

second dose is to be administered at eight months after the first dose. Preferably the second dose is administered at about eight-and-a-half to nine months after the first dose.

A vaccine composition according to the present invention may be administered in a single dose.

Dengue disease, as defined herein, may be caused by any one of two serotypes of a dengue virus. For example, dengue disease is preferably caused by a dengue virus of serotype 1 or serotype 3, a dengue virus of serotype 1 or serotype 4, a dengue virus of serotype 3 or serotype 4, a dengue virus of serotype 1 or serotype 2, a dengue virus of serotype 2 or serotype 3, a dengue virus of serotype 2 or serotype 4. Dengue disease, as defined herein, is preferably caused by any one of three serotypes of a dengue virus. For example, dengue disease is preferably caused by a dengue virus of serotype 1, 2 or 3, a dengue virus of serotype 1, 3 or 4, a dengue virus of serotype 1, 2 or 4, a dengue virus of serotype 2, 3 or 4. In another embodiment, dengue disease is caused by a dengue virus of serotype 1, a dengue virus of serotype 2, a dengue virus of serotype 3 or a dengue virus of serotype 4.

A vaccine composition according to the present invention, e.g. for use in a method according to the present invention preferably comprises a dengue antigen of serotype 1, a dengue antigen of serotype 2, a dengue antigen of serotype 3 and a dengue antigen of serotype 4. Such a composition may be described herein as a tetravalent composition. For instance, a composition of the present invention, e.g. for use in a method of protecting according to the present invention, may advantageously comprise any one of the following combinations of dengue antigens of serotypes 1, 2, 3 and 4: i) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-LAV, a chimeric dengue virus comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; ii) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-BID, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; (iii) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-PR, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; (iv) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-MD, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4;. For instance, a composition of the present invention may also advantageously comprise any one of the following combinations of dengue antigens:

i) CYD-1, CYD-LAV, CYD-3 and CYD-4; ii) CYD-1, CYD-BID, CYD-3 and CYD-4; (iii) CYD-1, CYD-PR, CYD-3 and CYD-4 or (iv) CYD-1, CYD-MD, CYD-3 and CYD-4. A composition of the present invention may also advantageously comprise the following combination of dengue antigens: i) a dengue antigen comprising the prM and E sequences of CYD-1, VDV2, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4. For instance, a composition of the present invention may advantageously comprise CYD-1, VDV-2, CYD-3 and CYD-4. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises a sequence having at least 90% identity to the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2. For example, said sequence may be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the prm-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2.

A vaccine composition according to the present invention, e.g. for use in a method according to the present invention, preferably comprises a dengue antigen of serotype 1, a dengue antigen of serotype 2, a dengue antigen of serotype 3 and a dengue antigen of serotype 4. Such a composition may be described herein as a tetravalent composition. For instance, a composition of the present invention, e.g. for use in a method of protecting according to the present invention, may advantageously comprise any one of the following combinations of dengue antigens of serotypes 1, 2, 3 and 4: i) a dengue antigen comprising the M and E sequences of CYD-1, a dengue antigen comprising the M and E sequences of CYD-LAV, a chimeric dengue virus comprising the M and E sequences of CYD-3 and a dengue antigen comprising the M and E sequences of CYD-4; ii) a dengue antigen comprising the M and E sequences of CYD-1, a dengue antigen comprising the M and E sequences of CYD-BID, a dengue antigen comprising the M and E sequences of CYD-3 and a dengue antigen comprising the M and E sequences of CYD-4; (iii) a dengue antigen comprising the M and E sequences of CYD-1, a dengue antigen comprising the M and E sequences of CYD-PR, a dengue antigen comprising the M and E sequences of CYD-3 and a dengue antigen comprising the M and E sequences of CYD-4; (iv) a dengue antigen comprising the M and E sequences of CYD-1, a dengue antigen comprising the M and E sequences of CYD-MD, a dengue antigen comprising the M and E sequences of

CYD-3 and a dengue antigen comprising the M and E sequences of CYD-4;. For instance, a composition of the present invention may also advantageously comprise any one of the following combinations of dengue antigens: i) CYD-1, CYD-LAV, CYD-3 and CYD-4; ii) CYD-1, CYD-BID, CYD-3 and CYD-4; (iii) CYD-1, CYD-PR, CYD-3 and CYD-4 or (iv) CYD-1, CYD-MD, CYD-3 and CYD-4. A composition of the present invention may also advantageously comprise the following combination of dengue antigens: i) a dengue antigen comprising the M and E sequences of CYD-1, VDV2, a dengue antigen comprising the M and E sequences of CYD-3 and a dengue antigen comprising the M and E sequences of CYD-4. For instance, a composition of the present invention may advantageously comprise CYD-1, VDV-2, CYD-3 and CYD-4. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises the E sequence of CYD-LAV (SEQ ID NO: 13), CYD-BID (SEQ ID NO: 14), CYD-PR (SEQ ID NO: 15) CYD-MD (SEQ ID NO: 16) or SEQ ID NO: 18. In certain embodiments, a composition of the present invention which comprises a dengue antigen comprising the sequence as set forth in SEQ ID NO: 18 is not a vaccine composition of serotype 2 comprising the prM-E sequence from SEQ ID NO: 2. In certain embodiments, a composition of the present invention is either a composition comprising a dengue antigen comprising the sequence as set forth in SEQ ID NO: 18, wherein said composition is not a vaccine composition of the present invention comprising chimeric dengue virus of serotype 2 generated using the prM-E sequence of SEQ ID NO: 2, or it is a composition comprising chimeric dengue virus of serotype 2 generated using the prM-E sequence of SEQ ID NO: 2. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises a sequence having at least 90% identity to the E sequence of CYD-LAV (SEQ ID NO: 13), CYD-BID (SEQ ID NO: 14), CYD-PR (SEQ ID NO: 15) CYD-MD (SEQ ID NO: 16) or SEQ ID NO: 18. For example, said sequence may be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the E sequence of CYD-LAV (SEQ ID NO: 13), CYD-BID (SEQ ID NO: 14), CYD-PR (SEQ ID NO: 15) CYD-MD (SEQ ID NO: 16) or SEQ ID NO: 18.

A composition of the present invention, as described herein, (e.g. a tetravalent formulation, e.g. for use in a method of the present invention), may advantageously comprise a dengue antigen of serotype 2 which comprises a polypeptide selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 or SEQ ID NO: 23. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a polypeptide having the sequence of SEQ ID NO: 19, said vaccine composition is preferably either: (i) a vaccine composition

comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 19, wherein said vaccine composition does not comprise CYD-LAV or (ii) a vaccine composition comprising CYD-LAV. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 19, wherein said vaccine composition does not comprise a dengue antigen comprising the M and E sequences of CYD-LAV or (ii) a vaccine composition comprising a dengue antigen comprising the M and E sequences of CYD-LAV. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 19, wherein said vaccine composition does not comprise a dengue antigen comprising the prM and E sequences of CYD-LAV or (ii) a vaccine composition comprising a dengue antigen comprising the prM and E sequences of CYD-LAV. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 19, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequence of LAV-2 or (ii) a vaccine composition comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequence of LAV-2 (SEQ ID NO: 8). Preferably, a vaccine composition of the present invention which comprises a polypeptide having the sequence of SEQ ID NO: 19 does not comprise: (i) a chimeric virus comprising the prM and E amino acid sequences of LAV-2; (ii) a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-LAV (SEQ ID NO: 8) or (iii) a dengue antigen comprising the prM-E sequence from LAV-2. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a polypeptide having the sequence of SEQ ID NO: 21, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 21, wherein said vaccine composition does not comprise CYD-PR or (ii) a vaccine composition comprising CYD-PR. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 21, wherein said vaccine composition does not comprise a dengue antigen comprising the prM and E sequences of CYD-PR or (ii) a vaccine composition comprising a dengue antigen comprising the prM and E sequences of CYD-PR. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 21, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequence of PR/DB023 (SEQ ID NO: 10) or (ii) a vaccine composition

comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequence of PR/DB023 (SEQ ID NO: 10). Preferably, a vaccine composition of the present invention which comprises a polypeptide having the sequence of SEQ ID NO: 21 does not comprise: (i) a chimeric virus comprising the prM and E amino acid sequences of PR/DB023; (ii) a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-PR (SEQ ID NO: 10) or (iii) a dengue antigen comprising the prM-E sequence from PR/DB023. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a polypeptide having the sequence of SEQ ID NO: 22, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 22, wherein said vaccine composition does not comprise CYD-MD or (ii) a vaccine composition comprising CYD-MD. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 22, wherein said vaccine composition does not comprise a dengue antigen comprising the prM and E sequences of CYD-MD or (ii) a vaccine composition comprising a dengue antigen comprising the prM and E sequences of CYD-MD. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 22, wherein said vaccine composition does not comprise a dengue antigen comprising the M and E sequences of CYD-MD or (ii) a vaccine composition comprising a dengue antigen comprising the M and E sequences of CYD-MD. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 22, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequence of MD1280 (SEQ ID NO: 11) or (ii) a vaccine composition comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequence of MD1280 (SEQ ID NO: 11). Preferably, a vaccine composition of the present invention which comprises a polypeptide having the sequence of SEQ ID NO: 22 does not comprise: (i) a chimeric virus comprising the prM and E amino acid sequences of MD1280; (ii) a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-MD (SEQ ID NO: 11) or (iii) a dengue antigen comprising the prM-E sequence from MD1280. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a polypeptide having the sequence of SEQ ID NO: 23, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 23, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype

2 generated using the prM-E sequence SEQ ID NO: 2 or (ii) a vaccine composition comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequence of SEQ ID NO: 2. Preferably, a vaccine composition of the present invention which comprises a polypeptide having the sequence of SEQ ID NO: 23 does not comprise: (i) a dengue antigen of serotype 2 which comprises SEQ ID NO: 2 or (ii) a dengue antigen comprising the prM-E sequence from SEQ ID NO: 2.

Preferably a dengue antigen of serotype 2 of the present invention further comprises a polypeptide selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 18. For instance, said dengue antigen of serotype 2 preferably comprises: i) a polypeptide of SEQ ID NO: 13 and a polypeptide of SEQ ID NO: 19; ii) a polypeptide of SEQ ID NO: 14 and a polypeptide of SEQ ID NO: 20; iii) a polypeptide of SEQ ID NO: 15 and a polypeptide of SEQ ID NO: 21; iv) a polypeptide of SEQ ID NO: 16 and a polypeptide of SEQ ID NO: 22; or v) a polypeptide of SEQ ID NO: 18 and a polypeptide of SEQ ID NO: 23. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a polypeptide having the sequence of SEQ ID NO: 13 and a polypeptide having the sequence of SEQ ID NO: 19, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 13 and a polypeptide having the sequence of SEQ ID NO: 19, wherein said vaccine composition does not comprise CYD-LAV or (ii) a vaccine composition comprising CYD-LAV. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 13 and a polypeptide having the sequence of SEQ ID NO: 19, wherein said vaccine composition does not comprise a dengue antigen comprising the prM and E sequences of CYD-LAV or (ii) a vaccine composition comprising a dengue antigen comprising the prM and E sequences of CYD-LAV. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 13 and a polypeptide having the sequence of SEQ ID NO: 19, wherein said vaccine composition does not comprise a dengue antigen comprising the M and E sequences of CYD-LAV or (ii) a vaccine composition comprising a dengue antigen comprising the M and E sequences of CYD-LAV. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 13 and a polypeptide having the sequence of SEQ ID NO: 19, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequence of LAV-2 or (ii) a vaccine composition comprising a chimeric dengue

virus of serotype 2 generated using the prM-E sequence of LAV-2 (SEQ ID NO: 8). Preferably, a vaccine composition of the present invention which comprises a polypeptide having the sequence of SEQ ID NO: 13 and a polypeptide having the sequence of SEQ ID NO: 19 does not comprise: (i) a chimeric virus comprising the prM and E amino acid sequences of LAV-2; (ii) a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-LAV (SEQ ID NO: 8) or (iii) a dengue antigen comprising the prM-E sequence from LAV-2. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a polypeptide having the sequence of SEQ ID NO: 15 and a polypeptide having the sequence of SEQ ID NO: 21, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 15 and a polypeptide having the sequence of SEQ ID NO: 21, wherein said vaccine composition does not comprise CYD-PR or (ii) a vaccine composition comprising CYD-PR. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen a polypeptide having the sequence of SEQ ID NO: 15 and comprising a polypeptide having the sequence of SEQ ID NO: 21, wherein said vaccine composition does not comprise a dengue antigen comprising the prM and E sequences of CYD-PR or (ii) a vaccine composition comprising a dengue antigen comprising the prM and E sequences of CYD-PR. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 15 and a polypeptide having the sequence of SEQ ID NO: 21, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequence of PR/DB023 (SEQ ID NO: 10) or (ii) a vaccine composition comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequence of PR/DB023 (SEQ ID NO: 10). Preferably, a vaccine composition of the present invention which comprises a polypeptide having the sequence of SEQ ID NO: 15 and a polypeptide having the sequence of SEQ ID NO: 21 does not comprise: (i) a chimeric virus comprising the prM and E amino acid sequences of PR/DB023; (ii) a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-PR (SEQ ID NO: 10) or (iii) a dengue antigen comprising the prM-E sequence from PR/DB023. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a polypeptide having the sequence of SEQ ID NO: 16 and a polypeptide having the sequence of SEQ ID NO: 22, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 16 and a polypeptide having the sequence of SEQ ID NO: 22, wherein said vaccine composition does not comprise CYD-MD or (ii) a vaccine

composition comprising CYD-MD. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a polypeptide having the sequence of SEQ ID NO: 16 and a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 22, wherein said vaccine composition does not comprise a dengue antigen comprising the prM and E sequences of CYD-MD or (ii) a vaccine composition comprising a dengue antigen comprising the prM and E sequences of CYD-MD. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 16 and a polypeptide having the sequence of SEQ ID NO: 22, wherein said vaccine composition does not comprise a dengue antigen comprising the M and E sequences of CYD-MD or (ii) a vaccine composition comprising a dengue antigen comprising the M and E sequences of CYD-MD. Said vaccine composition is also preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 16 and a polypeptide having the sequence of SEQ ID NO: 22, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequence of MD1280 (SEQ ID NO: 11) or (ii) a vaccine composition comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequence of MD1280 (SEQ ID NO: 11). Preferably, a vaccine composition of the present invention which comprises a polypeptide having the sequence of SEQ ID NO: 16 and a polypeptide having the sequence of SEQ ID NO: 22 does not comprise: (i) a chimeric virus comprising the prM and E amino acid sequences of MD1280; (ii) a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-MD (SEQ ID NO: 11) or (iii) a dengue antigen comprising the prM-E sequence from MD1280. When a vaccine composition of the present invention comprises a dengue antigen of serotype 2 which comprises a polypeptide having the sequence of SEQ ID NO: 18 and a polypeptide having the sequence of SEQ ID NO: 23, said vaccine composition is preferably either: (i) a vaccine composition comprising a dengue antigen comprising a polypeptide having the sequence of SEQ ID NO: 18 and a polypeptide having the sequence of SEQ ID NO: 23, wherein said vaccine composition does not comprise a chimeric dengue virus of serotype 2 generated using the prM-E sequence of SEQ ID NO: 2 or (ii) a vaccine composition comprising a chimeric dengue virus of serotype 2 generated using the prM-E sequence of SEQ ID NO: 2. Preferably, a vaccine composition of the present invention which comprises a polypeptide having the sequence of SEQ ID NO: 18 and a polypeptide having the sequence of SEQ ID NO: 23 does not comprise: (i) a dengue antigen of serotype 2 which comprises SEQ ID NO: 2 or (ii) a dengue antigen comprising the prM-E sequence from SEQ ID NO: 2.

A composition of the present invention, as described herein (e.g. a tetravalent formulation, e.g. for use a method of the present invention), may advantageously comprise a dengue antigen of serotype 2 which comprises a polypeptide having at least 90% identity to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 or SEQ ID NO: 23. Preferably said dengue antigen of serotype 2 further comprises a polypeptide having at least 90% identity to SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 18. For instance, said dengue antigen of serotype 2 preferably comprises: i) a polypeptide having at least 90% sequence identity to SEQ ID NO: 13 and a polypeptide having at least 90% sequence identity to SEQ ID NO: 19; ii) a polypeptide having at least 90% sequence identity to SEQ ID NO: 14 and a polypeptide having at least 90% sequence identity to SEQ ID NO: 20; iii) a polypeptide having at least 90% sequence identity to SEQ ID NO: 15 and a polypeptide having at least 90% sequence identity to SEQ ID NO: 21; iv) a polypeptide having at least 90% sequence identity to SEQ ID NO: 16 and a polypeptide having at least 90% sequence identity to SEQ ID NO: 22; or v) a polypeptide having at least 90% sequence identity to SEQ ID NO: 18 and a polypeptide having at least 90% sequence identity to SEQ ID NO: 23. In preferred embodiments, the references herein to at least 90% identity may be read as at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to the given sequence.

The dengue antigens of serotype 2 as described in the preceding paragraphs may advantageously be combined with any of the dengue antigens of serotypes 1, 3 and 4 as described elsewhere herein to form a tetravalent formulation comprising a dengue antigen of serotype 1, a dengue antigen of serotype 2, a dengue antigen of serotype 3 and a dengue antigen of serotype 4. For instance, the dengue antigens of serotypes 1, 3 and 4 may each be independently selected from the group consisting of a live attenuated dengue virus, an inactivated dengue virus, a live attenuated or inactivated chimeric dengue virus or a dengue virus-like particle (VLP). Preferably, said dengue antigens of serotype 1, 3 and 4 are each independently selected from the group consisting of a live attenuated dengue virus and a live attenuated chimeric dengue virus. Preferably, said dengue antigens of serotypes 1, 3 and 4 are live attenuated chimeric dengue viruses of serotypes 1, 3 and 4 respectively. Preferably, said live attenuated chimeric dengue viruses of serotypes 1, 3 and 4 each comprise one or more proteins from a dengue virus and one or more proteins from a different flavivirus. For example, each of said live attenuated chimeric dengue viruses of serotypes 1, 3 and 4 is advantageously a YF/Dengue chimera. Preferably, said dengue antigens of serotypes 1, 3 and 4 are each a live attenuated chimeric dengue virus in which the genetic backbone of a recipient

flavivirus has been modified by exchanging the sequences encoding the prM and E proteins of the recipient flavivirus with the corresponding sequences of a dengue virus. Preferably said recipient flavivirus is a yellow fever virus. For example, in an advantageous embodiment, said live attenuated chimeric dengue viruses of serotypes 1, 3 and 4 are respectively a Chimerivax dengue serotype 1 strain (i.e. a CYD-1 strain), a Chimerivax dengue serotype 3 strain (i.e. a CYD-3 strain) and a Chimerivax dengue serotype 4 strain (i.e. a CYD-4 strain).

It is an aim of the present inventors to provide an optimized tetravalent dengue vaccine composition (i.e. vaccine composition comprising a dengue antigen of each of serotypes 1, 2, 3 and 4) which provides an improved neutralising antibody response against dengue virus of serotype 2 when compared with the neutralising antibody response generated by CYD-1, CYD-2, CYD-3 and CYD-4 as defined in Example 1.

Accordingly, in one aspect, the present invention advantageously provides a vaccine composition which comprises a dengue antigen of each of serotypes 1, 2, 3 and 4, wherein said dengue antigens of serotypes 1, 3 and 4 are each a live attenuated chimeric dengue virus and said dengue antigen of serotype 2 is a live attenuated dengue virus which comprises a nucleic acid sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 24.

Accordingly, in another aspect, the present invention advantageously provides a vaccine composition which comprises a dengue antigen of serotype 1, a dengue antigen of serotype 2, a dengue antigen of serotype 3 and a dengue antigen of serotype 4, wherein:

i) said dengue antigen of serotype 1 is a YF/dengue chimeric dengue virus (i.e. a recipient yellow fever virus in which the genetic backbone of the YF virus has been modified by exchanging the sequences encoding the prM and E proteins of the YF virus by the corresponding sequences of a dengue serotype 1 virus);

ii) said dengue antigen of serotype 2 is a live attenuated dengue virus of serotype 2 which comprises a nucleic acid sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 24;

iii) said dengue antigen of serotype 3 is a YF/dengue chimeric dengue virus (i.e. a recipient yellow fever virus in which the genetic backbone of the YF virus has been modified by exchanging the sequences encoding the prM and E proteins of the YF virus by the corresponding sequences of a dengue serotype 3 virus) and

iv) said dengue antigen of serotype 4 is a YF/dengue chimeric dengue virus (i.e. a recipient yellow fever virus in which the genetic backbone of the YF virus has

been modified by exchanging the sequences encoding the prM and E proteins of the YF virus by the corresponding sequences of a dengue serotype 4 virus).

Preferably, said recipient YF virus (which forms the genetic backbone of the YF/dengue chimeric viruses of serotypes 1, 3 and 4) is an attenuated YF virus. For example, said recipient YF virus may be an attenuated YF virus selected from the group consisting of YF 17D, YF 17DD and YF 17D204. Preferably, the YF/dengue chimeric viruses of serotypes 1, 3 and 4 are respectively a Chimerivax dengue serotype 1 (i.e. a CYD-1), a Chimerivax dengue serotype 3 (i.e. a CYD-3) and a Chimerivax dengue serotype 4 (i.e. a CYD-4).

A reference herein to a nucleic acid sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 24 may preferably be read as a nucleic acid sequence having at least 92%, at least 94%, at least 96%, at least 98%, at least 99% or 100% sequence identity to the sequence as set forth in SEQ ID NO: 24. Preferably the nucleotides at the positions within said nucleic acid sequences (that have at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 24) which correspond to positions 736, 1619, 4723, 5062, 9191, 10063, 10507, 57, 524, 2055, 2579, 4018, 5547, 6599 and 8571 of SEQ ID NO: 24 are not mutated. Advantageously, a dengue antigen of serotype 2 which is a live attenuated dengue virus for use in a composition of the present invention (for example for use in combination with a dengue antigen of serotypes 1, 3 and 4 as described above and elsewhere herein (e.g. dengue antigens of serotypes 1, 3 and 4 which are live attenuated chimeric dengue viruses, e.g. YF/dengue chimeric dengue viruses)) is a live attenuated dengue virus which comprises a nucleic acid sequence having 100% sequence identity to the sequence as set forth in SEQ ID NO: 24 or a live attenuated dengue virus which comprises at least one and no more than 20 nucleotide substitutions when compared with the sequence as set forth in SEQ ID NO: 24. Preferably said live attenuated dengue virus comprises at least one and no more than 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3 or 2 nucleotide substitutions when compared with the sequence as set forth in SEQ ID NO: 24. Preferably the nucleotides at the positions within said nucleic acid sequences which correspond to positions 736, 1619, 4723, 5062, 9191, 10063, 10507, 57, 524, 2055, 2579, 4018, 5547, 6599 and 8571 of SEQ ID NO: 24 are not mutated. Advantageously, a dengue antigen of serotype 2 which is a live attenuated dengue virus for use in a composition of the present invention comprises a nucleic acid sequence that has no more than 20 base mutations, deletions or insertions when compared with the sequence as set forth in SEQ ID NO: 24. In certain cases said live attenuated dengue virus of serotype 2 comprises a nucleic acid sequence that has no more than 15 or even no more than 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 base

mutations, deletions or insertions when compared with the sequence as set forth in SEQ ID NO: 24. Preferably the nucleotides at the positions within said nucleic acid sequence that correspond to positions 736, 1619, 4723, 5062, 9191, 10063, 10507, 57, 524, 2055, 2579, 4018, 5547, 6599 and 8571 of SEQ ID NO: 24 are not mutated.

It is also preferred that a dengue antigen of serotype 2 for use in a vaccine composition of the present invention (e.g. a dengue antigen which is a live attenuated dengue virus or a live attenuated chimeric dengue virus of serotype 2) is capable of inducing neutralizing antibodies in humans and is capable of inducing a balanced immune response when used in the context of a tetravalent dengue vaccine composition. It is also preferred that a dengue antigen of serotype 2 for use in a vaccine composition of the present invention (e.g. a dengue antigen which is a live attenuated dengue virus or a live attenuated chimeric dengue virus of serotype 2) for use in a vaccine composition of the invention results in low or absent viremia in humans. It is also preferred that a dengue antigen of serotype 2 for use in a tetravalent vaccine composition of the present invention (e.g. a dengue antigen which is a live attenuated dengue virus or a live attenuated chimeric dengue virus of serotype 2) provides an improved neutralising antibody response against dengue virus of serotype 2 when compared with the neutralising antibody response generated by CYD-1, CYD-2, CYD-3 and CYD-4 as defined in Example 1.

Advantageously, a composition for use in the present invention comprises a dengue antigen of each of serotypes 1, 2, 3 and 4, wherein: (i) said dengue antigen of serotype 1 is a live attenuated chimeric dengue virus other than CYD-1 or said dengue antigen of serotype 1 is CYD-1; (ii) said dengue antigen of serotype 2 is a live attenuated dengue virus other than VDV-2 or said dengue antigen of serotype 2 is VDV-2; (iii) said dengue antigen of serotype 3 is a live attenuated chimeric dengue virus other than CYD-3 or said dengue antigen of serotype 3 is CYD-3 and (iv) said dengue antigen of serotype 4 is a live attenuated chimeric dengue virus other than CYD-4 or said dengue antigen of serotype 4 is CYD-4. In this context, the VDV-2 strain is the strain derived from the DEN-2 16681/PDK53 strain (LAV2) by subsequent adaptation to Vero cells, wherein said VDV-2 strain has 10 additional mutations in comparison with the DEN-2 16681/PDK53 strain including four silent mutations.

Advantageously, a composition for use in the present invention comprises a dengue antigen of each of serotypes 1, 2, 3 and 4, wherein said dengue antigens of serotypes 1, 3 and 4 are each a live attenuated chimeric dengue virus and said dengue antigen of serotype 2 is a live attenuated dengue virus which comprises a nucleic acid sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 24 and wherein said dengue antigens of serotypes 1, 2, 3 and 4 are not CYD-1, VDV-

2, CYD-3 and CYD-4 respectively or a dengue antigen comprising the M and E sequences of CYD-1, VDV2, a dengue antigen comprising the M and E sequences of CYD-3 and a dengue antigen comprising the M and E sequences of CYD-4 respectively.

Advantageously, a composition for use in the present invention comprises a dengue antigen of each of serotypes 1, 2, 3 and 4, wherein: (i) said dengue antigen of serotype 1 is a live attenuated chimeric dengue virus other than CYD-1 or said dengue antigen of serotype 1 is CYD-1; (ii) said dengue antigen of serotype 2 is a live attenuated dengue virus other than VDV-2 or said dengue antigen of serotype 2 is VDV-2; (iii) said dengue antigen of serotype 3 is a live attenuated chimeric dengue virus other than CYD-3 or said dengue antigen of serotype 3 is CYD-3 and (iv) said dengue antigen of serotype 4 is a live attenuated chimeric dengue virus other than CYD-4 or said dengue antigen of serotype 4 is CYD-4. In this context, the VDV-2 strain is the strain comprising the nucleic acid sequence as set forth in SEQ ID NO: 24.

A preferred vaccine composition according to the present invention, e.g. for use in a method according to the present invention, comprises a dengue antigen of serotype 1, a dengue antigen of serotype 2, a dengue antigen of serotype 3 and a dengue antigen of serotype 4, wherein:

i) said dengue antigen of serotype 1 is a YF/dengue chimeric dengue virus other than a CYD-1 or said dengue antigen of serotype 1 is a CYD-1;

ii) said dengue antigen of serotype 2 is a live attenuated dengue virus of serotype 2 which comprises a nucleic acid sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 24, wherein said dengue antigen of serotype 2 is not a live attenuated dengue virus of serotype 2 which comprises a nucleic acid sequence having 100% sequence identity to the sequence as set forth in SEQ ID NO: 24 or said dengue antigen of serotype 2 is a live attenuated dengue virus of serotype 2 which comprises a nucleic acid sequence having 100% sequence identity to the sequence as set forth in SEQ ID NO: 24;

iii) said dengue antigen of serotype 3 is a YF/dengue chimeric dengue virus other than a CYD-3 or said dengue antigen of serotype 3 is a CYD-3; and

iv) said dengue antigen of serotype 4 is a YF/dengue chimeric dengue virus other than a CYD-4 or said dengue antigen of serotype 4 is a CYD-4.

Advantageously, a dengue antigen of serotype 2 which is a live attenuated chimeric dengue virus for use in a vaccine composition of the present invention (for example for use in combination with a dengue antigen of serotypes 1, 3 and 4 as described above and elsewhere herein (e.g. dengue antigens of serotypes 1, 3 and 4

which are YF/dengue chimeric dengue viruses)) comprises a nucleic acid sequence having at least 90% identity to the sequence as set forth in SEQ ID NO: 25. Preferably said nucleic acid sequence has at least 92%, at least 94%, at least 96%, at least 98%, at least 99% or 100% sequence identity to the sequence as set forth in SEQ ID NO: 25. Preferably the nucleotides at the positions within said nucleic acid sequence which correspond to positions 524, 736, 1619 and 2055 of SEQ ID NO: 24 are not mutated (i.e. maintain the nucleotide appearing in SEQ ID NO: 24 at those positions).

Advantageously, a dengue antigen of serotype 2 which is a chimeric dengue virus for use in a vaccine composition of the present invention (for example for use in combination with a dengue antigen of serotypes 1, 3 and 4 as described above and elsewhere herein (e.g. dengue antigens of serotypes 1, 3 and 4 which are YF/dengue chimeric dengue viruses)) comprises a prM-E sequence having at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to the prM-E sequence from the LAV-2 strain (i.e. the RNA equivalent of SEQ ID NO: 4). Preferably the nucleotides at the positions within said prM-E sequence which correspond to positions 524, 736, 1619 and 2055 of the RNA equivalent of SEQ ID NO: 24 are not mutated (i.e. maintain the nucleotide appearing in the RNA equivalent of SEQ ID NO: 24 at those positions).

Advantageously, a dengue antigen of serotype 2 which is a chimeric dengue virus for use in a vaccine composition of the present invention (for example for use in combination with a dengue antigen of serotypes 1, 3 and 4 as described above and elsewhere herein (e.g. dengue antigens of serotypes 1, 3 and 4 which are YF/dengue chimeric dengue viruses)) comprises a prM-E sequence having at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to the prM-E sequence from the MD1280 strain (i.e. the RNA equivalent of SEQ ID NO: 7).

A composition of the present invention, as described herein, may advantageously comprise a dengue antigen selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus and (d) a combination of two or more of (a) to (c), wherein said dengue antigen comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 1.

A composition of the present invention, as described herein, may advantageously comprise a dengue antigen selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus and (d) a combination of two or more of (a) to (c), wherein said dengue antigen comprises a nucleotide sequence encoding M and E sequences as described herein.

For instance, a composition of the present invention, e.g. for use in a method of protecting according to the present invention, may advantageously comprise any one of the following combinations of dengue antigens of serotypes 1, 2, 3 and 4: i) CYD-1, CYD-LAV, CYD-3 and CYD-4; ii) CYD-1, CYD-BID, CYD-3 and CYD-4; (iii) CYD-1, CYD-PR, CYD-3 and CYD-4 or (iv) CYD-1, CYD-MD, CYD-3 and CYD-4. A composition of the present invention may also advantageously comprise the following combination of dengue antigens: i) a dengue antigen comprising the prM and E sequences of CYD-1, VDV2, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4. For instance, a composition of the present invention may advantageously comprise CYD-1, VDV-2, CYD-3 and CYD-4. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises a sequence having at least 90% identity to the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2. For example, said sequence may be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the prm-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2.

A vaccine composition for use the present invention, e.g. for use in a method according to the present invention preferably comprises a dengue antigen which is a vaccinal dengue virus. Such vaccinal dengue viruses include, for example, inactivated viruses, live attenuated viruses and live attenuated chimeric dengue viruses. Preferably, the vaccinal dengue viruses are live attenuated chimeric dengue viruses. Preferably, a live attenuated chimeric dengue virus according to the present invention comprises one or more proteins from a dengue virus and one or more proteins from a different flavivirus. Advantageously, said different flavivirus is a yellow fever virus, for example a yellow fever virus of strain YF 17D. Preferably a chimeric dengue virus according to the present invention comprises the prM-E amino acid sequences of a dengue virus, for example a chimeric dengue virus according to the present invention comprises a yellow fever virus genome whose prM-E whose prM-E sequence has been substituted with the prM-E sequence of a dengue virus. Advantageously, a vaccine composition according to the present invention, e.g. for use in a method of the present invention, comprises CYD-1, CYD-2, CYD-3 and CYD-4. A composition of the present invention may advantageously

comprise any one of the following combinations of dengue antigens i) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-LAV, a chimeric dengue virus comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; ii) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-BID, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; (iii) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-PR, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4; (iv) a dengue antigen comprising the prM and E sequences of CYD-1, a dengue antigen comprising the prM and E sequences of CYD-MD, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4;. For instance, a composition of the present invention may also advantageously comprise any one of the following combinations of dengue antigens: i) CYD-1, CYD-LAV, CYD-3 and CYD-4; ii) CYD-1, CYD-BID, CYD-3 and CYD-4; (iii) CYD-1, CYD-PR, CYD-3 and CYD-4 or (iv) CYD-1, CYD-MD, CYD-3 and CYD-4. A composition of the present invention may also advantageously comprise the following combination of dengue antigens: i) a dengue antigen comprising the prM and E sequences of CYD-1, VDV2, a dengue antigen comprising the prM and E sequences of CYD-3 and a dengue antigen comprising the prM and E sequences of CYD-4. For instance, a composition of the present invention may advantageously comprise CYD-1, VDV-2, CYD-3 and CYD-4. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2. Advantageously, a vaccine composition of the present invention, e.g. a chimeric dengue virus, of serotype 2 may comprise prM-E sequences having at least 90%, at least 95%, at least 98% or at least 99% identity to the prM-E sequences from the serotype 2 strains LAV-2, BID-V585, PR/DB023 or MD1280 as described in the examples or may comprise prM-E sequences having at least 90%, at least 95%, at least 98% or at least 99% identity to the prM-E sequence shown in SEQ ID NO: 2. Advantageously, a vaccine composition, e.g. a chimeric dengue virus, of serotype 2 for use in the method of the present invention may comprise prM-E sequences from the serotype 2 strains LAV-2, BID-V585, PR/DB023 or MD1280 or the prM-E sequence from SEQ ID NO: 2 as described in the examples. When the recipient genomic backbone of such chimeric dengue viruses is derived from YF-VAX®, such strains are referred to

herein as CYD-LAV, CYD-BID, CYD-PR and CYD-MD. A vaccine composition of the present invention comprising chimeric dengue virus of serotype 2 generated using the prM-E sequences of the serotype 2 strains LAV-2 (SEQ ID NO: 8), BID-V585 (SEQ ID NO: 9), PR/DB023 (SEQ ID NO: 10), MD1280 (SEQ ID NO: 11) or SEQ ID NO: 2, or generated using prM-E sequences having at least 90%, at least 95%, at least 98% or at least 99% identity to the prM-E sequences from the serotype 2 strains LAV-2, BID-V585, PR/DB023, MD1280 or the prM-E sequence from SEQ ID NO: 2 may advantageously be used in combination with CYD-1, CYD-3 and CYD-4 in a vaccine composition according to the present invention. A composition of the present invention, as described herein, may advantageously comprise a dengue antigen of serotype 2 which comprises a sequence having at least 90% identity to the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2. For example, said sequence may be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the prM-E sequence of CYD-LAV (SEQ ID NO: 8), CYD-BID (SEQ ID NO: 9), CYD-PR (SEQ ID NO: 10) CYD-MD (SEQ ID NO: 11) or SEQ ID NO: 2.

The exact quantity of a vaccinal dengue virus of the present invention to be administered may vary according to the age and the weight of the patient being vaccinated, the frequency of administration as well as the other ingredients (e.g. adjuvants) in the composition. The quantity of a live attenuated dengue virus of each of serotypes 1 to 4 comprised in a vaccine composition of the present invention lies within a range of from about 10^3 to about 10^7 $CCID_{50}$. Generally, the quantity of a live attenuated dengue virus of each of serotypes 1 to 4 comprised in a vaccine composition of the present invention lies within a range of from about 10^3 to about 10^6 $CCID_{50}$, for example within a range of from about 5×10^3 to about 5×10^5 $CCID_{50}$, for example within a range of from about 1×10^4 to about 1×10^5 $CCID_{50}$, for example about 10^5 $CCID_{50}$. The quantity of a live attenuated dengue virus of each of serotypes 1 to 4 comprised in a vaccine composition of the present invention may also lie within a range of from about 10^4 to about 10^7 $CCID_{50}$, for example about 10^6 $CCID_{50}$. The quantity of a live attenuated dengue virus of each of serotypes 1 to 4 comprised in a tetravalent composition of the present invention may be equal. For example a tetravalent composition of the present invention may comprise about 10^5 $CCID_{50}$ of each live attenuated dengue virus of serotypes 1 to 4. Alternatively, a tetravalent composition of the present invention may comprise about 10^6 $CCID_{50}$ of each live attenuated dengue virus of serotypes 1 to 4. Generally, the quantity of an inactivated dengue virus of each of serotypes 1 to 4 comprised in a composition of the present invention lies within a range of from about 10^4 to about 10^8 $CCID_{50}$ equivalent,

preferably within a range of from about 5 x 10^4 to about 5 x 10^7 $CCID_{50}$ equivalent, preferably within a range of from about 1 x 10^4 to about 1 x 10^6 $CCID_{50}$ equivalent, preferably about 10^5 $CCID_{50}$ equivalent. Generally, the quantity of a VLP of each of serotypes 1 to 4 comprised in the composition lies within a range of from about 100ng to about 100µg of VLP, preferably within a range of from about 100ng to about 50µg, preferably within a range of from about 100ng to about 20µg, preferably about 1µg to 10µg. The amount of VLP can be determined by ELISA. Advantageously, a vaccine composition according to the present invention comprises an effective amount of a dengue antigen as defined herein.

A vaccine composition according to the present invention may further comprise a pharmaceutically acceptable carrier or excipient. A pharmaceutically acceptable carrier or excipient according to the present invention means any solvent or dispersing medium etc., commonly used in the formulation of pharmaceuticals and vaccines to enhance stability, sterility and deliverability of the active agent and which does not produce any secondary reaction, for example an allergic reaction, in humans. The excipient is selected on the basis of the pharmaceutical form chosen, the method and the route of administration. Appropriate excipients, and requirements in relation to pharmaceutical formulation, are described in “Remington's Pharmaceutical Sciences” (19th Edition, A.R. Gennaro, Ed., Mack Publishing Co., Easton, PA (1995)). Particular examples of pharmaceutically acceptable excipients include water, phosphate-buffered saline (PBS) solutions and a 0.3% glycine solution. A vaccine composition according to the present invention may advantageously comprise 0.4% saline and 2.5% human serum albumin (HSA).

A vaccine composition for use in a method of the present invention may optionally contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, human serum albumin, essential amino acids, nonessential amino acids, L-arginine hydrochlorate, saccharose, D-trehalose dehydrate, sorbitol, tris (hydroxymethyl) aminomethane and/or urea. In addition, the vaccine composition may optionally comprise pharmaceutically acceptable additives including, for example, diluents, binders, stabilizers, and preservatives. Preferred stabilizers are described in WO 2010/003670.

A vaccine composition of the present invention may comprise a dengue antigen which is a dengue immunoprotein. A dengue immunoprotein, as used herein, is a dengue envelope (E) protein, or derivative or fragment thereof, that when administered to an immunocompetent subject induces neutralizing antibodies against a dengue virus of

serotype 1, 2, 3 or 4. Dengue immunoproteins include native and derivatized forms of dengue E proteins, including chemical conjugates, immunological fragments, and fusion proteins thereof.

Dengue immunoproteins, or derivatives or fragments thereof may be conjugated to carrier molecules. Such conjugation may be achieved by chemical conjugation techniques or through the recombinant expression of fusion proteins comprising the dengue immunoproteins or derivatives or fragments thereof and the carrier molecule. Examples of carrier molecules which may be used for preparing conjugates include diphtheria toxoid, tetanus toxoid, fragment C of tetanus toxin, mutants of diphtheria toxin including CRM 197, CRM 176, CRM228, CRM 45, CRM 9, CRM 45, CRM 102, CRM 103 and CRM 107, pneumococcal pneumolysin, OMPC, heat shock proteins, pertussis proteins, pneumococcal surface protein PspA or the toxin A or B of *Clostridium difficile*.

A vaccine composition of the present invention may comprise one or more adjuvants to enhance the immunogenicity of the dengue antigens. Those skilled in the art will be able to select an adjuvant which is appropriate in the context of this invention. An adjuvant is preferably used in a vaccine composition of the invention comprising an inactivated virus or a VLP or a dengue structural protein. An adjuvant may be used in a vaccine composition of the invention comprising a live attenuated virus, as long as said adjuvant does not impact replication.

Suitable adjuvants include an aluminum salt such as aluminum hydroxide gel, aluminum phosphate or alum, but may also be a salt of calcium, magnesium, iron or zinc. Further suitable adjuvants include an insoluble suspension of acylated tyrosine or acylated sugars, cationically or anionically derivatized saccharides, or polyphosphazenes. Alternatively, the adjuvant may be an oil-in-water emulsion adjuvant (EP 0 399 843B), as well as combinations of oil-in-water emulsions and other active agents (WO 95/17210; WO 98/56414; WO 99/12565 and WO 99/11241). Other oil emulsion adjuvants have been described, such as water-in-oil emulsions (U.S. Pat. No. 5,422, 109; EP 0 480 982 B2) and water-in-oil-in-water emulsions (U.S. Pat. No. 5,424,067; EP 0 480 981 B). Examples of such adjuvants include MF59, AF03 (WO 2007/006939), AF04 (WO 2007/080308), AF05, AF06 and derivatives thereof. The adjuvant may also be a saponin, lipid A or a derivative thereof, an immunostimulatory oligonucleotide, an alkyl glucosamide phosphate, an oil in water emulsion or combinations thereof. Examples of saponins include Quil A and purified fragments thereof such as QS7 and QS21.

As appreciated by skilled artisans, a vaccine composition of the present invention is suitably formulated to be compatible with the intended route of administration. Examples of suitable routes of administration include for instance intramuscular,

transcutaneous, subcutaneous, intranasal, oral or intradermal. Advantageously, the route of administration is subcutaneous.

The vaccine compositions of the present invention may be administered using conventional hypodermic syringes or safety syringes such as those commercially available from Becton Dickinson Corporation (Franklin Lakes, NJ, USA) or jet injectors. For intradermal administration, conventional hypodermic syringes may be employed using the Mantoux technique or specialized intradermal delivery devices such as the BD Soluvia(TM) microinjection system (Becton Dickinson Corporation, Franklin Lakes, NJ, USA), may be used.

The volume of a vaccine composition of the present invention administered will depend on the method of administration. In the case of subcutaneous injections, the volume is generally between 0.1 and 1.0 ml, preferably approximately 0.5 ml.

Optionally, booster administrations of a vaccine composition according to the present invention may be used, for example between six months and ten years, for example six months, one year, three years, five years or ten years after initial immunization (i.e. after administration of the last dose scheduled in the initial immunization regimen).

According to one embodiment, the invention also provides a kit comprising a vaccine composition of the invention and instructions for the use of said vaccine composition in a method of protecting a human subject against dengue disease. The kit can comprise at least one dose (typically in a syringe) of any vaccine composition contemplated herein. According to one embodiment the kit may comprises a multi-dose formulation (typically in a vial) of any vaccine composition as described herein. The kit further comprises a leaflet mentioning the use of the said vaccine composition for the prevention of dengue disease or the use of the said vaccine for the prophylaxis of dengue disease. The leaflet may further mention the vaccination regimen and the human subject population to be vaccinated.

The efficacy of a vaccine composition of the present invention in reducing the likelihood or severity of dengue disease may be measured in a number of ways. For instance the efficacy of a vaccine composition of the present invention in reducing the likelihood or severity of symptomatic virologically-confirmed dengue disease may be calculated by measuring after the administration of at least one dose of said vaccine composition (e.g. after administration of one, two or three doses of said vaccine composition):

(i) the percentage of symptomatic virologically-confirmed dengue cases caused by dengue virus of any serotype;

(ii) the percentage of severe virologically-confirmed dengue cases caused by dengue virus of any serotype;

(iii) the percentage of dengue hemorrhagic fever cases of Grades I to IV caused by dengue virus of any serotype;

(iv) the percentage of DHF cases of Grade I caused by dengue virus of any serotype;

(v) the percentage of DHF cases of Grade II caused by dengue virus of any serotype;

(vi) the percentage of DHF cases of Grade III caused by dengue virus of any serotype;

(vii) the percentage of DHF cases of Grade IV caused by dengue virus of any serotype;

(viii) the annual incidence rate of hospitalized virologically-confirmed dengue caused by dengue virus of any serotype; and/or

(ix) the length of hospital stay for symptomatic virologically-confirmed dengue cases caused by dengue virus of any serotype;

in a group of subjects that has received said vaccine composition and comparing those measurements with the equivalent measurements from a control group of subjects that has not received said vaccine composition, wherein the subjects in both said groups are resident in a Dengue endemic region. A statistically significant reduction in any one or more of (i) to (ix) in the vaccinated group of subjects when compared with the unvaccinated control group of subjects is indicative of the efficacy of a vaccine composition according to the present invention. In a preferred embodiment, the efficacy of a vaccine composition according to the present invention is demonstrated by a statistically significant reduction of one or more of the measures as described above, wherein the DHF cases or dengue cases are caused by dengue virus of serotypes 1, 3 or 4.

The efficacy of a vaccine composition according to the present invention in reducing the severity or likelihood of dengue disease may also be calculated by measuring after the administration of at least one dose of said vaccine composition (e.g. after administration of one, two or three doses of said vaccine composition):

(i) the mean duration and/or intensity of fever;

(iii) the mean value for plasma leakage as defined by a change in haematocrit;

(iii) the mean value for thrombocytopenia (platelet count); and/or

(iv) the mean value of the level of liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST);

in a group of subjects that has received said vaccine composition and who have developed virologically-confirmed dengue disease and comparing those measurements with the equivalent measurements from a control group of subjects that has not received said vaccine composition and who have developed virologically-confirmed dengue disease. A statistically significant reduction in any one or more of (i) to (v) in the vaccinated group of subjects who have developed virologically-confirmed dengue disease when compared with the control group of subjects who have developed virologically-confirmed dengue disease is indicative of the efficacy of a vaccine composition according to the present invention in reducing the severity or likelihood of dengue disease.

Typically the efficacy of the method of protection of the invention against a dengue disease, as measured e.g. by the method described in example 1 ($VE=100*(1-ID_{CYD}/ID_{Control})$, where ID is the incidence density (i.e., the number of human subjects with virologically-confirmed dengue divided by the number of person-years at risk) in each group), is at least 50%, preferably at least 60%, wherein said dengue disease is caused by serotype 1, 3 or 4. The efficacy of the method of protection being advantageously at least 70%, preferably 80% against a dengue disease caused by serotype 3 or 4. The efficacy of the method of protection being advantageously at least 90% against dengue disease caused by serotype 4.

Percent identity between two amino acid sequences or two nucleotide sequences is determined by standard alignment algorithms such as, for example, Basic Local Alignment Tool (BLAST) described in Altschul et al. (1990) J. Mol. Biol., 215: 403-410, the algorithm of Needleman et al. (1970) J. Mol. Biol., 48: 444-453; the algorithm of Meyers et al. (1988) Comput. Appl. Biosci., 4: 11-17; or Tatusova et al. (1999) FEMS Microbiol. Lett., 174: 247-250, etc. Such algorithms are incorporated into the BLASTN, BLASTP and "BLAST 2 Sequences" programs (see www.ncbi.nim.nih.gov/BLAST). When utilizing such programs, the default parameters can be used. For example, for nucleotide sequences the following settings can be used for "BLAST 2 Sequences" : program BLASTN, reward for match 2, penalty for mismatch-2, open gap and extension gap penalties 5 and 2 respectively, gap x~dropoff 50, expect 10, word size 11, filter ON. For amino acid sequences the following settings can be used for "BLAST 2 Sequences" : program BLASTP, matrix BLOSUM62, open gap and extension gap penalties 11 and 1 respectively, gap x~dropoff 50, expect 10, word size 3, filter ON.

It is understood that the various features and preferred embodiments of the present invention as disclosed herein may be combined together.

Throughout this application, various references are cited. The disclosures of these references are hereby incorporated by reference into the present disclosure.

The present invention will be further illustrated by the following examples. It should be understood however that the invention is defined by the claims, and that these examples are given only by way of illustration of the invention and do not constitute in any way a limitation thereof.

EXAMPLES

Example 1: One year follow-up in Thailand of patients vaccinated with a tetravalent dengue vaccine (TDV) composition comprising Chimerivax™ DEN-1, DEN-2, DEN-3 and DEN-4

Methods

Study design and participants

An observer-blind, randomised, controlled, monocentre, Phase IIb trial of the efficacy of the tetravalent Chimerivax™ vaccine (i.e. a tetravalent vaccine comprising the particular CYD-1 strain generated from the prM and E sequences of DEN1 PU0359 (TYP 1 140), the particular CYD-2 strain generated from the prM and E sequences of DEN2 PUO218, the particular CYD-3 strain generated from the prM and E sequences of DEN3 PaH881/88 and the particular CYD-4 strain generated from the prM and E sequences of DEN4 1228 (TVP 980), see WO 03/101397 and Guy *et al.*, Vaccine (2011), 29(42): 7229-41) against virologically-confirmed dengue disease is conducted. 4002 schoolchildren aged 4–11 years who are in good health based on medical history and physical examination are enrolled into the trial. The study is conducted at Ratchaburi Regional Hospital (RRH), Ratchaburi province, Muang district, Thailand. Children with acute febrile illness at enrolment, those with congenital or acquired immunodeficiency, and those receiving immunosuppressive therapy or other prohibited treatments or vaccines are excluded. Participants are randomly assigned 2:1 to receive three doses of dengue vaccine or a control product at Months 0, 6 and 12.

Products

Each of the chimeric viruses are produced and cultured on Vero cells as described in WO 03/10197, Guy et al, Hum. Vaccines (2010) 6 (9): 696; Guy et al, Vaccine (2010) 28: 632; Guirakhoo et al, J. Virol. (2000) 74 : 5477 ; Guirakhoo et al, J. Virol. (2001) 75 (16) : 7290 ; Guirakhoo et al, Virol. (June 20, 2002) 298: 146; and Guirakhoo et al, J. Virol. (2004) 78 (9): 4761.

The vaccine is presented as a lyophilized powder (previously stored at temperature of between 2°C and 8°C), which is reconstituted with 0.5 mL of solvent for injection (0.4% NaCl containing 2.5% human serum albumin). As reconstituted, each 0.5 mL dose of vaccine contains 5 ± 1 $\log_{10}$ $CCID_{50}$ of each chimeric dengue serotype (1, 2, 3 and 4) and excipients (essential amino acids, non-essential amino acids, L-arginine chlorhydrate, saccharose, D-trehalose dehydrate, sorbitol, tris (hydroxymethyl) aminoethane and urea). The control product is inactivated rabies vaccine (Verorab®, Sanofi Pasteur, Lyon France) for the first injection of the first 50 children randomised to the control group, and 0·9% NaCl saline placebo for all other injections. All products are injected subcutaneously into the deltoid region of the upper arm.

Assessments

All children are actively followed to detect acute febrile illness based on daily surveillance of school registers during school terms for absenteeism (followed by phone calls or home visits to absentees), and twice-weekly home visits, phone calls or mobile phone text-messages throughout school holidays. In any case of febrile illness (defined as illness with two temperature readings of ≥37.5°C at least 4 hours apart) parents are asked to take their child to RRH for diagnosis and treatment. The surveillance system also captures spontaneous consultations at RRH. Consecutive febrile episodes separated by a symptom-free interval of ≥14 days are considered as separate episodes. Paired serum samples are collected at presentation (i.e., acute sample, collected no later than 7 days after fever onset) and 7–14 days later (convalescent sample) and sent to Sanofi Pasteur's Global Clinical Immunology (GCI) laboratory (Swiftwater, PA, USA) and to the Centre for Vaccine Development (CVD, Mahidol University, Thailand). Acute samples are screened for the presence of flavivirus using an initial RT-PCR assay which detects the presence of any flavivirus (using primers composed of highly conserved flavivirus sequences). Positive samples are tested for wild-type dengue virus with a serotype-specific quantitative RT-PCR, as described herein. In parallel, all acute samples are tested for the presence of dengue NS1 antigen using commercial ELISA kit (Platelia™, Bio-Rad Laboratories, Marnes-La-Coquette, France). A virologically-confirmed episode of dengue disease is defined as a positive result in either the serotype-specific RT-PCR, or the NS1 antigen ELISA.

Active surveillance is maintained until each participant has been followed for at least 13 months after the third vaccination and until the Independent Data Monitoring Committee (IDMC) confirms that ≥27 cases have occurred in the per-protocol (PP) population.

All serious adverse events (SAE) are documented until the sixth month after the last vaccination, and thereafter any fatal SAE or vaccine-related SAE.

Dengue immune responses are assessed in the first 300 enrolled children at RRH in sera collected at enrolment and 28 days after each injection. Sera are also prospectively collected from all participants on Day 28 after the third injection to assess immune responses in children with virologically-confirmed dengue occurring from this timepoint. Sera are sent to GCI for measurement of serotype-specific neutralizing antibody titres against the CYD parental dengue viruses using the plaque-reduction neutralization test ($PRNT_{50}$) as described herein. The assay's quantitation limit is 10 (1/dil). Samples below this value are assigned the titre 5 and considered seronegative.

Statistical analysis

The primary objective is to determine vaccine efficacy (VE) against cases of symptomatic, virologically-confirmed dengue occurring more than 28 days after the third vaccination among children enrolled and vaccinated as planned, according to the equation: $VE = 100*(1-ID_{CYD}/ID_{Control})$, where ID is the incidence density (i.e., the number of children with virologically-confirmed dengue divided by the number of person-years at risk) in each group. With an assumed disease incidence of 1·3%, a true VE of 70%, a minimum follow-up period of 1 year after the third vaccination, and a per protocol (PP) subject attrition rate of 7·5%/year, 4002 subjects assigned with a 2:1 ratio to dengue vaccine or control are needed to demonstrate, with more than 82% power, and 95% confidence, that VE is not nul. Analyses are based on the two-sided 95% confidence interval (CI) of VE, calculated using the Exact method (Breslow NE, Day NE. Statistical Methods in Cancer Research, Volume II – The Design and Analysis of Cohort Studies. International Agency for Research on Cancer (IARC scientific publication No. 82), Lyon, France). The primary analysis is performed on the PP population, i.e. those who satisfy the enrolment criteria, who correctly receive all three doses of the assigned vaccine at Months 0, 6 (±15 days), and 12 (±30 days), and for whom group allocation is not unmasked. This analysis is repeated on the full analysis set for efficacy, in those who receive three injections. As a secondary objective, VE against dengue is determined before completion of the 3-dose vaccination regimen. In an analysis defined after unblinding, VE against each serotype individually is investigated. Analyses for safety and immunogenicity endpoints are descriptive, using 95%CI.

Results

Of the 4002 children enrolled, 95.9% complete the vaccinations and 91.8% are included in the per protocol (PP) analysis set for efficacy. Vaccine and control groups are comparable for age and gender. More than 90% of those sampled at baseline are positive for antibodies against dengue or JEV.

Efficacy

During the study, 131 dengue cases (131 children had 136 episodes) are virologically-confirmed. Of these, 77 occur more than 28 days after the third injection in the PP population and are included in the primary analysis: 45 cases occurred during 2522 person-years at risk in the vaccine group, while 32 cases occurred during 1251 person-years at risk in the control group. The corresponding vaccine efficacy is 30·2% (95%CI: −13·4–56·6). This finding is confirmed in the full analysis set (see Table 1 below). Efficacy after at least one injection is 33·4% (95%CI: 4·1–53·5) and after at least two injections is 35·3% (95%CI: 3·3–56·5).

Table 1: Serotype-specific and overall efficacy of CYD tetravalent dengue vaccine against virologically-confirmed dengue disease

	Dengue vaccine		Control		Efficacy	
	Person-years at risk	Cases or Episodes*	Person-years at risk	Cases or Episodes*	%	(95% CI)
>28 days after 3 injections (per-protocol analysis)						
Cases	2522	45	1251	32	30·2	(−13·4–56·6)
Serotype 1 episodes	2536	9	1251	10	55·6	(-21·6-84·0)
Serotype 2 episodes	2510	31	1250	17	9·2	(-75·3-51·3)
Serotype 3 episodes	2541	1	1257	2	75·3	(-375·0-99·6)
Serotype 4 episodes	2542	0	1263	4	100	(24·8-100)
NS1 Antigen positive only episodes	2542	4	1265	0	ND	ND
>28 days after 3 injections (Full analysis set)						
Cases	2620	46	1307	34	32·5	(−8·5–57·6)
Serotype 1 episodes	2633	9	1308	10	55·3	(−22·5–83·9)
Serotype 2 episodes	2608	32	1307	19	15·6	(−57·6–53·6)
Serotype 3 episodes	2638	1	1312	2	75·1	(−378–99·6)
Serotype 4 episodes	2641	0	1320	4	100	(−24·3–100)
NS1 Antigen positive only episodes	2640	4	1322	0	ND	ND
>28 days after at least 1 injection (Full analysis set)						
Cases	5089	75	2532	56	33·4	(4·1–53·5)
Serotype 1 episodes	5139	14	2564	18	61·2	(17·4–82·1)
Serotype 2 episodes	5107	51	2560	26	1·7	(−64·3–39·8)

Serotype 3 episodes	5144	4	2565	10	80·1	(30·9–95·4)
Serotype 4 episodes	5149	1	2577	5	90·0	(10·5–99·8)
NS1 Antigen positive only episodes	5147	5	2579	1	−150·5	(−11750–72·0)

Data are number except where indicated. ND: not determined. *A 'case' was defined as a first episode of dengue fever virologically-confirmed by either serotype-specific PCRs, or NS1 antigen ELISA. Serotype-specific efficacy was calculated including all episodes of that serotype; 5 children with two virologically confirmed dengue episodes during the study were therefore included twice in the serotype-specific analysis.

Post-hoc analyses reveal differing efficacy by serotype (see Table 1). Efficacy against DENV1, DENV3, and DENV4 after at least one injection is in the range 61·2%–90·0%, compared with 1·7% against DENV2. Efficacy against DENV1, DENV3, and DENV4 after three injections is in the range 55.3%–100%, compared with 15.6% against DENV2.

In those subjects that acquired virologically-confirmed dengue, a statistically significant reduction in the annual incidence rate of hospitalization was observed in the vaccinated group when compared with the control group. The relative risk (RR) after three doses was 0.523 (see Table 2).

Table 2: Incidence of hospitalized virologically-confirmed dengue during the trial

Time period	CYD Dengue Vaccine Group (N=2666) M	Cases	Annual Incidence Rate (95%CI)	n Occurrences	Control Group (N=1331) M	Cases	Annual Incidence Rate (95%CI)	n Occurrences	Relative Risk RR	(95%CI)
Year 1	2666	8	0.3 (0.1; 0.6)	8	1331	7	0.5 (0.2; 1.1)	7	0.571	(0.181, 1.85)
Year 2	2557	24	0.9 (0.5; 1.3)	24	1282	23	1.7 (1.0; 2.5)	23	0.523	(0.283, 0.970)

Year 1 = D0 to injection 3 ; Year 2 = Injection 3 to the end of Active Phase

Table 3: Rate of hospitalisation by serotype

	Vaccinee Group (%)	Control Group (%)
Serotype 1	8/14 (57.1)	9/18 (50%)
Serotype 2	20/52 (38.5)	15/27 (55.6)
Serotype 3	1/4 (25)	3/11 (27.3)
Serotype 4	0/1	2/5 (40)

No serotype NS1 +ve	3/5 (60)	1/1 (100)
Total	32/76 (42.1)	30/62 (48.4)

Immunogenicity

Geometric mean titres (GMT) of neutralising antibodies against dengue serotypes 1–4 on Day 28 after the third injection in the per-protocol analysis set are, respectively, 146 (95%CI: 98·5–217), 310 (224–431), 405 (307–534), and 155 (123–196) in the vaccine group. In the control group these values are 23·9 (14·0–40·9), 52·2 (26·8–102), 48·9 (25·5–93·9), and 19·4 (11·6–32·2). Post one year GMTs are respectively 76.5; 122; 94 and 153 for serotypes 1, 2, 3 and 4.

Safety

There are 584 SAEs during this phase of the study: 366 are reported by 11.8% (315/2666) of participants in the vaccine group, and 218 are reported by 13.2% (176/1331) of participants in the control group. There are no vaccine-related SAEs in the dengue group and there is one in the control group. SAEs observed are medical conditions consistent with the age group and showed no clustering within the 7- or 28-day post-vaccination periods.

Virologically-confirmed dengue cases occurring as a breakthrough in vaccinees were not more serious than those cases occurring in the control group.

Sequence of the prM-E region of circulating wild type serotype 2 strain in the trial

The nucleotide and amino acid sequence of the prM-E region of the wild type serotype 2 strain that causes the DEN-2 cases in the trial is determined. These are set out below as SEQ ID NO: 1 and SEQ ID NO: 2 respectively. The E and the M amino acid sequences of the serotype 2 strain that causes the DEN-2 cases in the trial are described in SEQ ID NOs: 18 and 23 respectively.

>nucleotide sequence (SEQ ID NO: 1)

ttccatctaaccacacgcaacggagaaccacacatgatcgtcggtatacaggagaaaggga
aaagtcttctgttcaaaacagaggatggtgtgaacatgtgcaccctcatggctatggacct
tggtgaattgtgtgaagacacaatcacgtacaagtgtcctcttctcaggcagaatgagcca
gaagacatagactgttggtgcaactccacgtccacgtgggtaacctatgggacctgtacca

ctacgggagaacataggagagaaaaaagatcagtggcactcgttccacatgtgggaatggg
actggagacgcgaaccgaaacatggatgtcatcagaaggggcttggaaacatgcccagaga
attgaaacttggatcctgagacatccaggcttcaccataatggcagcaatcctggcataca
ccataggaacgacacatttccagagagtcctgattttcatcctactgacagctgtcgctcc
ttcaatgacaatgcgttgcataggaatatcaaatagagactttgtagaaggggtttcagga
ggaagttgggttgacatagtcttagaacatggaagctgtgtgacgacgatggcaaaaaaca
aaccaacattggatttcgaactgataaaaacggaagccaaacagcctgccaccctaaggaa
gtactgcatagaagcaaaactaaccaacacaacaacagaatcccgttgcccaacacaaggg
gaacccagcctaaaagaagagcaggacaagaggttcgtctgcaaacactccatggtagaca
gaggatggggaaatggatgtggattatttggaaagggaggcattgtgacctgtgctatgtt
cacatgcaaaaagaacatggaagggaaaatcgtgcaaccagaaaacttggaatacaccatt
gtggtaacacctcactcaggggaagagcatgcggtcggaaatgacacaggaaaacacggca
aggaaatcaaagtaacaccacagagttccatcacagaagcagaactgacaggttatggcac
cgtcacgatggagtgctccccgagaacaggcctcgacttcaatgagatggtgttgctgcag
atggaaaataaagcttggctggtgcataggcaatggtttctagacctgccattaccatggc
tgcccggagcggataaacaagaatcaaattggatacagaaagaaacattggtcactttcaa
aaatccccatgcgaagaaacaggatgttgttgttttaggatcccaagaaggggccatgcat
acagcactcacaggagccacagaaatccaaatgtcgtcaggaaacttgctcttcactggac
atctcaagtgcaggctgagaatggacaagctacagcttaaaggaatgtcatactctatgtg
cacaggaaagtttaaagttgtgaaggaaatagcagaaacacaacatggaacgatagttatc
agagtgcaatatgaaggggacggctctccatgtaaaattccttttgagataatggatttgg
aaaaaagatatgtcttaggccgcctgatcacagtcaacccaattgtaacagaaaaagacag
cccagtcaacatagaagcagaacctccattcggagacagttacatcatcataggagtagag
ccgggacaactgaagctcaactggttcaagaaaggaagtttctatcggccaaatgtttgaga
caacgatgagaggggcgaagagaatggccattttgggtgacacagcctgggacttcggatc
cctgggaggagtgtttacatctataggaaaagctctccaccaagtctttggagcgatctat
ggggctgccttcagtggggtttcatggaccatgaaaatcctcataggagtcattatcacat
ggataggaatgaactcacgcagcacctcactgtctgtgtcactggtactggtgggaattgt
gacactgtatttaggagtcatggtgcaggcc

>amino acid sequence (SEQ ID NO: 2)

FHLTTRNGEPHMIVGIQEKGKSLLFKTEDGVNMCTLMAMDLGELCEDTITYKCPLLRQNEP
EDIDCWCNSTSTWVTYGTCTTTGEHRREKRSVALVPHVGMGLETRTETWMSSEGAWKHAQR
IETWILRHPGFTIMAAILAYTIGTTHFQRVLIFILLTAVAPSMTMRCIGISNRDFVEGVSG
GSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTESRCPTQG
EPSLKEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENLEYTI
VVTPHSGEEHAVGNDTGKHGKEIKVTPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQ
MENKAWLVHRQWFLDLPLPWLPGADKQESNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMH
TALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGKFKVVKEIAETQHGTIVI
RVQYEGDGSPCKIPFEIMDLEKRYVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYIIIGVE
PGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIY
GAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGIVTLYLGVMVQA

>amino acid sequence (SEQ ID NO: 18)

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCI
EAKLTNTTTESRCPTQGEPSLKEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCK
KNMEGKIVQPENLEYTIVVTPHSGEEHAVGNDTGKHGKEIKVTPQSSITEAELTGYGTVTM
ECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADKQESNWIQKETLVTFKNPH

AKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGK
FKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRYVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGG
VFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGIVTLY
LGVMVQA

>amino acid sequence (SEQ ID NO: 23)

SVALVPHVGMGLETRTETWMSSEGAWKHAQRIETWILRHPGFTIMAAILAYTIGTTHFQRV
LIFILLTAVAPSMT

Discussion

The main finding from this study is that a safe, efficacious vaccine against dengue based on the chimeric CYD viruses is possible. Estimated efficacy against DENV1, 3 and 4 is in a range consistent with the 70% hypothesis and is statistically significant after at least one vaccination. Efficacy in a range consistent with the 70% hypothesis is not observed against DENV2. Since DENV2 is the prevalent serotype in this study, overall vaccine efficacy is diminished in this setting.

The vaccine's safety and reactogenicity profile is good, and no vaccine-related SAEs and no safety signals are identified during the review of AEs and SAEs collected from over two years of active follow-up of more than 2600 vaccinees. Theoretical safety concerns associated with the potential enhancement of the rate or severity of dengue disease by an incomplete immune response against the four serotypes of dengue have previously hampered vaccine development. In this trial, the absence of disease enhancement in the presence of an incomplete immune response against the circulating DENV2 viruses is an important and reassuring finding. For instance, cases in vaccinees do not differ from cases in controls in terms of factors such as the duration of fever or in terms of the classical clinical signs of dengue such as bleeding, plasma leakage or thrombocytopenia. Furthermore, severe dengue was not more frequent among vaccinees than controls at any point during the trial).

It was also demonstrated that, in those subjects that acquired virologically-confirmed dengue, a statistically significant reduction in the annual incidence rate of hospitalization was observed in the vaccinated group when compared with the control group. This reduction was seen in those subjects that acquired virologically-confirmed dengue of serotype 2 (see Table 3).

The results observed in respect of DENV2 may be explained by a number of contributing factors. For instance, there is a possible antigenic mismatch between the CYD2 vaccine virus and the DENV2 virus that causes disease in the trial. In the 1990s,

the Asian 1 genotype of DENV2 emerged in South-East Asia, replacing the previously dominant Asian/American lineage of viruses. Several mutations identified in Domain 2 of the E protein (E83, and in particular E226 and E228) are suggestive of changing viral fitness and antigenicity. The donor wild-type virus for the CYD2 vaccine (and the challenge strain used in the PRNT50) was a clinical isolate from Bangkok in 1980 (Guirakhoo F et al., J Virol 2000, 74: 5477–85). While this virus is also classified as belonging to the Asian I genotype, the above-mentioned key amino acid residues in this virus (and thus in CYD2) correspond to those of the Asian/American genotype (Hang et al PLoS Negl Trop Dis. 2010 Jul 20;4(7):e757).

. Additionally, there are two extremely rare mutations in the prM-E sequence of the CYD2 vaccine that may also contribute to a mismatched immune response. These mutations are at positions prM24 and E251 (Guirakhoo et al, J. Virol. (2004) 78 (9): 4761).

The results observed against DENV2 are not associated with an absence of immunogenicity in the $PRNT_{50}$ assay. Neutralising antibody responses after vaccination against DENV2 are higher than those against DENV1 and DENV3.

In conclusion, the present study constitutes the first ever demonstration that a safe and efficacious dengue vaccine is possible and represents a major milestone in dengue vaccine development.

Example 2: Identification of optimized dengue vaccinal strains of serotype 2

The objective of the present example is to identify dengue virus strains of serotype 2 which provide the basis for generating optimized dengue vaccine compositions against dengue virus of serotype 2, wherein said optimized dengue vaccine compositions provide improved efficacy in comparison to Chimerivax™ CYD-2 when used in a method according to the present invention.

Criteria determining the selection of optimized strains for the determination of a universal dengue 2 antigen include: (i) recently circulating strain; (ii) balanced selection between Asian and American strains; (iii) an optimized strain should have a prM-E sequence that is as similar as possible to a calculated global consensus sequence generated by aligning the available prM-E sequences of dengue viruses of serotype 2; (iv) amino acid variations that are predicted to impact antibody recognition should be avoided; (v) rare amino acids at a particular positions in the prM and E sequences should be avoided, especially in the E protein ectodomain (a rare amino acid at a particular position is defined as a amino acid that appears at that position in less than 15% of the aligned sequences); (vi) optimized strains for which some previous laboratory experience exists

are preferred and (vii) a dengue antigen that leads to a balanced immune response in a tetravalent composition.

Criteria determining the selection of optimized strains for a local dengue 2 antigen (i.e. that is especially effective against a wild type dengue virus circulating in a particular area) are criteria (i) and (vii).

Methods

Databases

Sequence are retrieved from the National Center for Biotechnology Information (NCBI) Dengue virus variation database (www.ncbi.nlm.nih.gov/genomes/VirusVariation/Database/nph-select.cgi?tax_id=12637).

Sequence analyses

Sequence alignments are performed using the MUSCLE algorithm (Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res, 32(5):1792-1797).

Sequence alignment outputs are generated in Vector NTi version 9, module AlignX (Invitrogen). Sequence similarity searches are carried out using the BLAST algorithm (Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) Basic local alignment search tool. J Mol Biol, 215(3):403-410).

Sequence numbering for prM-E sequences

The sub-sequences included in the prM-E sequences may be numbered in various ways: (i) the total prM-E protein sequence is numbered from position 1 to position 661, with the preM protein sequence designated as position 1 to position 90/91, the M protein sequence designated as position 91/92 to position 166 and the E protein sequence designated as position 167 to position 661; (ii) the prM and M protein sequences are numbered together, i.e. from position 1 to position 166 of the total sequence and E is numbered separately from position 1 to position 495; (iii) the prM, M and E sequences are numbered separately, i.e. prM is numbered from position 1 to 90/91, M is numbered from 1 to 75/76 and E from position 1 to position 495.

Results

Public sequences retrieval

All available dengue virus serotype 2 full length prM and E protein sequences are downloaded from the NCBI Dengue database. Download of sequences takes place on two separate occasions - on 4 October 2010 and in 2011. On the first occasion 669 sequences are downloaded and on the second occasion approximately 3200 sequences are downloaded.

Global consensus sequence generation

On each occasion, all retrieved protein sequences are aligned to generate a global consensus sequence for the prM and E proteins of dengue virus of serotype 2. By definition, the global consensus sequence is an artificial sequence containing the most frequently encountered amino acid at each position. The global consensus sequences for the 2010 alignment and the 2011 alignment only differ by two amino acids. In the 2010 alignment, the global consensus sequence contains isoleucine and valine at positions 129 and 308 respectively of the E protein (by reference to the 1-495 E sequence numbering) and, by contrast, in the 2011 alignment, the global consensus sequence contains valine and isoleucine at positions 129 and 308 respectively of the E protein (by reference to the 1-495 E sequence numbering). The differences in the 2010 and 2011 global consensus sequences is explained by the fact that the respective percentages of strains containing valine or isoleucine at those positions is close to 50%. The global consensus sequence for the prM-E sequence is therefore represented as follows:

fhlttrngephmivgrqekgksllfktedgvnmctlmaidlgelcedtitykcpllrqnep
edidcwcnststwvtygtctttgehrrekrsvalvphvgmgletrtetwmssegawkhvqr
ietwilrhpgftimaailaytigtthfqralifilltavapsmtMRCIGISNRDFVEGVSG
GSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTESRCPTQG
EPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGK**X**VQPENLEYTI
VITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQ
MEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMH
TALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGKFK**Z**VKEIAETQHGTIVI
RVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYIIIGVE
PGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIY
GAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA (SEQ ID
NO: 3)

The global consensus sequence for the E sequence is represented as follows:

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCI
EAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCK
KNMEGK**X**VQPENLEYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTM
ECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPH
AKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGK
FK**Z**VKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGG
VFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLY
LGVMVQA (SEQ ID NO: 12)

In the above sequence, the global consensus prM sequence is shown in lower case letters and the E sequence is shown in upper case letters. The amino acid positions denoted as X (position 129 of the E sequence) and Z (position 308 of the E sequence) are each independently Val or Ile, i.e. the proportion of aligned amino acid sequences including Val or Ile at those positions is close to 50%.

Determination of minor amino acid residues and analysis of the Chimerivax™ CYD2 sequence

A list of variable amino acid positions is established from the global alignment containing all amino acid positions varying in at least 5% of the aligned sequences. In addition, any amino acid from the sequence of the prM and E proteins of Chimerivax™ CYD2 that do not match the global consensus sequence are also identified. The results are shown in Table 4 (N.B., in the table, the prM and M protein sequences are numbered together, i.e. from position 1 to position 166 of the total sequence and E is numbered separately from position 1 to position 495).

prM

Position	Consensus	%	CYD	%	Other variants
15	G	76	S	24	I<1
16	R	76			A16; D4
24	L	99	V	<1	
29	D	91			N8; E-V-H<1
31	V	94			T4; I2; M-D<1
39	I	58	M	40	L2
52	K	91			N9; T<1
55	L	93			F7; R<1
57	R	93			K8
82	T	90			A9; S1; I-V<1
120	V	55	N	43	A45
125	T	99	I	<1	N-S<1
127	I	94			V6; F<1
134	T	95			A5; I-S<1
148	H	90			Y9; N-D<1
152	A	70			V28; T1; I<1

M protein

Stem domain of E

E

Position	Consensus	%	CYD	%	Other variants
52	Q	83			H15; E2; L<1
61	I	93			V6; M-K-F-T<1
71	E	76			A19; D5; P<1
83	N	73			K25; V1; S-A-D<1
91	V	67			I31; L1
129	I	50	V	50	F-T<1
131	Q	83			L17; E-H-P<1
141	I	72	V	28	L<1
149	H	80			N19; Y-R-Q-P-S-T<1
160	K	94			Q3; E2; M1; R-N<1
162	I	94			V6; L<1
164	I	55	V	45	
203	D	49	N	46	E4; S1; K-G<1
226	T	84			K16; I-E-P<1
228	G	86			E14
251	V	99	F	<1	I<1
308	V	52			I48; L<1
340	M	80			T19; I-A-L<1
346	H	74			Y26; Q-L<1
359	T	95			A4; I2; M-P<1
462	I	78			V22; T<1
484	V	69			I31; F-A-L-T<1
485	V	94			I6; L<1
491	V	62			A38; G-L<1

Table 4: Dengue virus serotype 2 variable residues and CYD2 comparison

A total of 41 amino acid positions are identified in the prM and E sequences which either vary from the global consensus sequence in at least 5% of the aligned sequences and/or differ from the sequence of the prM and E proteins in CYD2. Ten amino acid positions in the sequence of the prM and E proteins in CYD2 differ from the global consensus sequence (5 positions in E, 2 positions in M and 3 in its precursor part, see Table 4). Five out of the ten differing residues present a variation distribution close to 50:50, suggesting a naturally variable position. Only three positions in the CYD2 prM-E sequence appear as very minor variants (pr-24 Val, M-125 Ile and E-251 Phe).

Impact analysis of variations in the E and M proteins

To gain further insight into the variable positions, changes in the E protein ectodomain (amino acids 1-395), the most important domain for the seroneutralisation by the immune system are further analysed.

Using information available from a published 3D structure of the soluble ectodomain of the E protein of a dengue virus of serotype 2 (Modis, Y., et al. (2003) Proc Natl Acad Sci U S A, 100(12):6986-6991), a 3D model of the Dengue virus particle surface is reconstructed. This allows a fine tuned assessment of the accessibility of each amino acid from the E ectodomain, which in turn is used in association with the variability level and the nature of the amino acid change to assess a potential impact of CYD2 variations on antibody recognition.

The analysis demonstrates that two variations in the Chimerivax™ CYD2 sequence from the global consensus sequence (Val 141 and Val 164 of the E protein) are completely buried in the 3D structure and so cannot directly interact with an antibody at the surface of the virion. Position 129 of the E protein is a 50:50 variable amino acid position between Val (Chimerivax™ CYD2) and Ile (global consensus sequence) and the substitution is also a fully conservative change. The potential impact of these variations is therefore considered as very limited.

The variation at position 203 of the E protein (Asn in Chimerivax™ CYD2 and Asp in the global consensus sequence) could potentially have an impact (well exposed residue, change of charge) but the distribution of the variation among strains is close to 50:50, suggesting a naturally variable position.

The variation at position 251 of the E protein of Chimerivax™ CYD2 (Phe in Chimerivax™ CYD2 and Val in the global consensus sequence) is extremely rare among retrieved strains. Such a variation could have some impact on recognition by an antibody, as it is rare, rather well exposed at the surface of the virion (29%) and corresponds to a non-conservative amino acid change.

The modeling analysis described above identifies two other position variations in the E protein that could have a potential impact on antibody recognition (positions 226 and 228), although Chimerivax™ CYD2 does not vary from the global consensus sequence at those positions. Therefore in identifying optimised serotype 2 strains, variations from the global consensus sequence at those positions (i.e. Thr at position 226 and glycine at position 228) are preferably avoided for a universal dengue 2 vaccine.

Without being bound by theory, the present inventors consider that the impact of amino acid variations in dengue virus sequences can also be assessed using a scoring method which takes into account a number of relevant factors. In particular this method takes into account the genome location of the variation (G), the nature of the amino acid change (B), 3D mapping (M) and known variants at the position in question (DB), wherein the score is calculated as G x B x M x DB. A score of 0 would be classified as no expected impact, a score of >0 to 10 would be classified as a low expected impact, a

score of >10 to 25 would be classified as a median expected impact and a score of >25 would be classified as a high expected impact.

The genome location (G) score is 0 if the amino acid is located in the M part of the prM/M protein (i.e. position 92 to 166 of the prM/M sequence) or in position 396 to 495 of the E protein. The genome location score is 1 if the amino acid is located in prM part of the prM/M protein (i.e. position 1 to 91 of the prM/M sequence) or in position 1 to 395 of the E protein.

The score related to the nature of the amino acid change (B) is calculated as B = 100 – [(Blosum95 score + 6) x 10], wherein the Blosum95 score for different amino acid substitutions is as shown in Table 5 below.

Table 5

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	*
A		-2	-2	-3	-1	-1	-1	-1	-3	-2	-2	-1	-2	-3	-1	1	0	-4	-3	-1	-3	-1	-1	-6
R	-2		-1	-3	-5	0	-1	-4	-1	-4	-3	2	-2	-4	-3	-2	-2	-4	-3	-4	-2	-1	-2	-6
N	-2	-1		1	-4	0	-1	-1	0	-4	-5	0	-3	-4	-3	0	-1	-5	-3	-4	4	-1	-2	-6
D	-3	-3	1		-5	-1	1	-2	-2	-5	-5	-2	-5	-5	-3	-1	-2	-6	-5	-5	4	0	-2	-6
C	-1	-5	-4	-5		-4	-6	-5	-5	-2	-3	-5	-3	-3	-5	-2	-2	-4	-4	-2	-4	-5	-3	-6
Q	-1	0	0	-1	-4		2	-3	1	-4	-3	1	-1	-4	-2	-1	-1	-3	-3	-3	-1	4	-1	-6
E	-1	-1	-1	1	-6	2		-3	-1	-4	-4	0	-3	-5	-2	-1	-2	-5	-4	-3	0	4	-2	-6
G	-1	-4	-1	-2	-5	-3	-3		-3	-6	-5	-3	-4	-5	-4	-1	-3	-5	-5	-5	-2	-3	-3	-6
H	-3	-1	0	-2	-5	1	-1	-3		-4	-4	-1	-3	-2	-3	-2	-2	-3	1	-4	-1	0	-2	-6
I	-2	-4	-4	-5	-2	-4	-4	-6	-4		1	-4	1	-1	-4	-3	-2	-4	-2	3	-5	-4	-2	-6
L	-2	-3	-5	-5	-3	-3	-4	-5	-4	1		-3	2	0	-4	-3	-2	-3	-2	0	-5	-4	-2	-6
K	-1	2	0	-2	-5	1	0	-3	-1	-4	-3		-2	-4	-2	-1	-1	-5	-3	-3	-1	0	-1	-6
M	-2	-2	-3	-5	-3	-1	-3	-4	-3	1	2	-2		-1	-3	-3	-1	-2	-3	0	-4	-2	-2	-6
F	-3	-4	-4	-5	-3	-4	-5	-5	-2	-1	0	-4	-1		-5	-3	-3	0	3	-2	-5	-4	-2	-6
P	-1	-3	-3	-3	-5	-2	-2	-4	-3	-4	-4	-2	-3	-5		-2	-2	-5	-5	-4	-3	-2	-3	-6
S	1	-2	0	-1	-2	-1	-1	-1	-2	-3	-3	-1	-3	-3	-2		1	-4	-3	-3	-1	-1	-1	-6
T	0	-2	-1	-2	-2	-1	-2	-3	-2	-2	-2	-1	-1	-3	-2	1		-4	-3	-1	-1	-2	-1	-6
W	-4	-4	-5	-6	-4	-3	-5	-5	-3	-4	-3	-5	-2	0	-5	-4	-4		2	-3	-6	-4	-4	-6
Y	-3	-3	-3	-5	-4	-3	-4	-5	1	-2	-2	-3	-3	3	-5	-3	-3	2		-3	-4	-4	-2	-6
V	-1	-4	-4	-5	-2	-3	-3	-5	-4	3	0	-3	0	-2	-4	-3	-1	-3	-3		-5	-3	-2	-6
B	-3	-2	4	4	-4	-1	0	-2	-1	-5	-5	-1	-4	-5	-3	-1	-1	-6	-4	-5		0	-2	-6
Z	-1	-1	-1	0	-5	4	4	-3	0	-4	-4	0	-2	-4	-2	-1	-2	-4	-4	-3	0		-1	-6
X	-1	-2	-2	-2	-3	-1	-2	-3	-2	-2	-2	-1	-2	-2	-3	-1	-1	-4	-2	-2	-2	-1		-6
*	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	

B = Asx, Z = Glx, X = Any and * = Stop

The M value depends on whether the amino acid is or is not located at the prM/E interface. For example, for CYD2 as used in Example 1, the amino acids that are located at the interface are prM residues 6, 7, 39, 40, 46-54, 56, 59-65, 67, 74 and 77 and E residues 64-72, 82-84, 101-104, 106-108 and 244-247. Where an amino acid is located at the interface, M equals 1. Where an amino acid is not located at the interface, M = Y x

SAS %. Y is 1 if the amino acid is located in an "up" position (i.e. directed towards the external environment); Y is 0.5 if the amino acid is located on the "side" of the molecule (i.e. the amino acid is neither directed towards the external environment nor towards the capsid) and Y is 0 if the amino acid is located in a "down" position (i.e. directed towards the capsid). The solvent accessibility surface % (SAS %) value is generated using the Discovery Studio 3D modeling software (Accelrys, Inc., CA, USA).

The DB value is 0 when the amino acid substitution results in an amino acid at the substitution position which is the most common amino acid at that position in the dengue sequences present in the GenBank database (http://www.ncbi.nlm.nih.gov). The DB value is 0.25 when the amino acid substitution results in an amino acid at the substitution position which is found in more than 5% of the dengue sequences present in the database (but is not the most common amino acid at that position). The DB value is 0.50 when the amino acid substitution results in an amino acid at the substitution position which is found in less than 5% of the dengue sequences present in the database (except unique substitutions). The DB value is 1 when the substitution amino acid is unique.

During replication, viruses may acquire a mutation leading to an amino acid substitution. The above-mentioned method provides a means to determine the effect of such mutations on the progeny of the mutated viruses.

Preferred sequences (i.e. sequences that are considered to be satisfactorily close to the identified consensus sequence) may have: (i) at most two, preferably one or no high-impact amino acid substitutions; (ii) at most three, preferably two or one, or no median impact amino acid substitutions; and/or (iii) at most five, four, three, two or one low impact amino acid substitutions.

Identification of optimized serotype 2 strains

Optimised serotype 2 strains are identified on the basis of the selection criteria described above.

A BLAST search is conducted to identify the strain having the closest sequence to the prM-E global consensus sequence in all of the available sequences. No sequence that is 100% identical to the prM-E global consensus sequence is found, but the best hit is a sequence from strain BID-V585 (NCBI Protein ID no. ACA58343; Genome ID no. EU529706; isolated from Puerto Rico in 2006) which shows only one variation from the global consensus sequence, at position 91 (Val in the global consensus sequence and Ile in BID-V585). The BID-V585 prM-E sequence contains 13 variations from the Chimerivax™ CYD-2 prM-E sequence.

A further strain selection is made so as to provide geographical balance in strain origin. Therefore a recently isolated Asian strain showing a good score in the BLAST analysis (strain MD-1280; NCBI Protein ID no. CAR65175; Genome ID no. FM21043; isolated from Viet Nam in 2004) is selected. Despite showing 6 variations with the global consensus sequence across prM-E, 3 of the 6 variations are identified as versatile positions naturally varying in more than 30% of the strains. The MD-1280 prM-E sequence contains 15 variations from the Chimerivax™ CYD-2 prM-E sequence.

A further strain selection is made on the basis of a large amount of previously accumulated experience with the strain. It is the PDK53-16681 strain, also known as the LAV-2 strain, a live-attenuated virus derived from Dengue serotype 2 16681 strain from Mahidol University (NCBI Protein ID no. AAA73186; Genome ID no. M84728; isolated from Thailand in 1964; Blok,J., et al. (1992); Virology 187 (2), 573-590). The LAV-2 prM-E sequence contains 10 variations from the global consensus sequence and 13 variations from the Chimerivax™ CYD-2 prM-E sequence.

A further strain selected on the basis of the above-mentioned criteria is strain PR/DB023 (NCBI Protein ID no. AEN71248; Genome ID no. JF804036; isolated from Puerto Rico in 2007). The PR/DB023 prM-E sequence contains 3 variations from the global consensus sequence and 13 variations from the Chimerivax™ CYD-2 prM-E sequence.

None of the selected strains contain the rare amino acids present in the Chimerivax™ CYD-2 prM-E sequence, i.e. Val at pr-24, Ile at M-125 and Phe at E-251.

PrM to E nucleotide sequences of the four selected strains

>LAV-2 prME nucleotide sequence (SEQ ID NO: 4)

ttccatttaaccacacgtaacggagaaccacacatgatcgtcagcagacaagagaaaggga
aaagtcttctgtttaaaacagaggttggcgtgaacatgtgtaccctcatggccatggacct
tggtgaattgtgtgaagacacaatcacgtacaagtgtccccttctcaggcagaatgagcca
gaagacatagactgttggtgcaactctacgtccacgtgggtaacttatgggacgtgtacca
ccatgggagaacatagaagagaaaaaagatcagtggcactcgttccacatgtgggaatggg
actggagacacgaactgaaacatggatgtcatcagaaggggcctggaaacatgtccagaga
attgaaacttggatcttgagacatccaggcttcaccatgatggcagcaatcctggcataca
ccataggaacgacacatttccaaagagccctgattttcatcttactgacagctgtcactcc
ttcaatgacaATGCGTTGCATAGGAATGTCAAATAGAGACTTTGTGGAAGGGGTTTCAGGA
GGAAGCTGGGTTGACATAGTCTTAGAACATGGAAGCTGTGTGACGACGATGGCAAAAAACA
AACCAACATTGGATTTTGAACTGATAAAAACAGAAGCCAAACAGCCTGCCACCCTAAGGAA
GTACTGTATAGAGGCAAAGCTAACCAACACAACAACAGAATCTCGCTGCCCAACACAAGGG
GAACCCAGCCTAAATGAAGAGCAGGACAAAAGGTTCGTCTGCAAACACTCCATGGTAGACA
GAGGATGGGGAAATGGATGTGGACTATTTGGAAAGGGAGGCATTGTGACCTGTGCTATGTT
CAGATGCAAAAAGAACATGGAAGGAAAAGTTGTGCAACCAGAAAACTTGGAATACACCATT

GTGATAACACCTCACTCAGGGGAAGAGCATGCAGTCGGAAATGACACAGGAAAACATGGCA
AGGAAATCAAAATAACACCACAGAGTTCCATCACAGAAGCAGAATTGACAGGTTATGGCAC
TGTCACAATGGAGTGCTCTCCAAGAACGGGCCTCGACTTCAATGAGATGGTGTTGCTGCAG
ATGGAAAATAAAGCTTGGCTGGTGCACAGGCAATGGTTCCTAGACCTGCCGTTACCATGGT
TGCCCGGAGCGGACACACAAGGGTCAAATTGGATACAGAAAGAGACATTGGTCACTTTCAA
AAATCCCCATGCGAAGAAACAGGATGTTGTTGTTTTAGGATCCCAAGAAGGGGCCATGCAC
ACAGCACTTACAGGGGCCACAGAAATCCAAATGTCATCAGGAAACTTACTCTTCACAGGAC
ATCTCAAGTGCAGGCTGAGAATGGACAAGCTACAGCTCAAAGGAATGTCATACTCTATGTG
CACAGGAAAGTTTAAAGTTGTGAAGGAAATAGCAGAAACACAACATGGAACAATAGTTATC
AGAGTGCAATATGAAGGGGACGGCTCTCCATGCAAGATCCCTTTTGAGATAATGGATTTGG
AAAAAAGACATGTCTTAGGTCGCCTGATTACAGTCAACCCAATTGTGACAGAAAAAGATAG
CCCAGTCAACATAGAAGCAGAACCTCCATTTGGAGACAGCTACATCATCATAGGAGTAGAG
CCGGGACAACTGAAGCTCAACTGGTTTAAGAAAGGAAGTTCTATCGGCCAAATGTTTGAGA
CAACAATGAGGGGGGCGAAGAGAATGGCCATTTTAGGTGACACAGCCTGGGATTTTGGATC
CTTGGGAGGAGTGTTTACATCTATAGGAAAGGCTCTCCACCAAGTCTTTGGAGCAATCTAT
GGAGCTGCCTTCAGTGGGGTTTCATGGACTATGAAAATCCTCATAGGAGTCATTATCACAT
GGATAGGAATGAATTCACGCAGCACCTCACTGTCTGTGACACTAGTATTGGTGGGAATTGT
GACACTGTATTTGGGAGTCATGGTGCAGGCC

UPPERCASE: E coding sequence; lowercase: prM coding sequence

> BID/V585 – prME nucleotide sequence (SEQ ID NO: 5)

ttccatttaaccacacgtaatggagaaccacacatgatcgttggtaggcaagagaaaggga
aaagtcttctgtttaaaacagaggatggtgttaacatgtgcaccctcatggccatagacct
tggtgaattgtgtgaagatacaatcacgtacaagtgccccctcctcaggcaaaatgaacca
gaagacatagattgttggtgcaactctacgtccacatgggtaacttatgggacatgtacca
ccacaggagaacacagaagagaaaaaagatcagtggcactcgttccacatgtgggcatggg
actggagacacgaactgaaacatggatgtcatcagaaggggcctggaaacatgttcagaga
attgaaacctggatcttgagacatccaggctttaccataatggcagcaatcctggcatata
ccataggaacgacacatttccaaagggctctgatcttcattttactgacagccgttgctcc
ttcaatgacaATGCGTTGCATAGGAATATCAAATAGAGACTTCGTAGAAGGGGTTTCAGGA
GGAAGTTGGGTTGACATAGTCTTAGAACATGGAAGTTGTGTGACGACGATGGCAAAAAATA
AACCAACATTGGATTTTGAACTGATAAAAACAGAAGCCAAACAACCTGCCACTCTAAGGAA
GTACTGTATAGAAGCAAAGCTGACCAATACAACAACAGAATCTCGTTGCCCAACACAAGGG
GAACCCAGTCTAAATGAAGAGCAGGACAAAAGGTTCATCTGCAAACACTCCATGGTAGACA
GAGGATGGGGAAATGGATGTGGATTATTTGGAAAGGGAGGCATTGTGACCTGTGCTATGTT
CACATGCAAAAAGAACATGGAAGGAAAAGTCGTGCAGCCAGAAAATCTGGAATACACCATC
GTGATAACACCTCACTCAGGAGAAGAGCACGCTGTAGGTAATGACACAGGAAAGCATGGCA
AGGAAATCAAAATAACACCACAGAGCTCCATCACAGAAGCAGAACTGACAGGCTATGGCAC
TGTCACGATGGAGTGCTCTCCGAGAACGGGCCTCGACTTCAATGAGATGGTACTGCTGCAG
ATGGAAGACAAAGCTTGGCTGGTGCACAGGCAATGGTTCCTAGACCTGCCGTTACCATGGC
TACCCGGAGCGGACACACAAGGATCAAATTGGATACAGAAAGAGACGTTGGTCACTTTCAA
AAATCCCCACGCGAAGAAACAGGACGTCGTTGTTTTAGGATCTCAAGAAGGGGCCATGCAC
ACGGCACTTACAGGGGCCACAGAAATCCAGATGTCATCAGGAAACTTACTGTTCACAGGAC
ATCTCAAGTGTAGGCTGAGAATGGACAAATTACAGCTTAAAGGAATGTCATACTCTATGTG
TACAGGAAAGTTTAAAATTGTGAAGGAAATAGCAGAAACACAACATGGAACAATAGTTATC
AGAGTACAATATGAAGGGGACGGCTCTCCATGTAAGATTCCTTTTGAGATAATGGATTTGG
AAAAAAGACACGTCCTAGGTCGCCTGATTACAGTGAACCCAATCGTAACAGAAAAAGATAG
CCCAGTCAACATAGAAGCAGAACCTCCATTCGGAGACAGCTACATCATCATAGGAGTAGAG
CCGGGACAATTGAAACTCAATTGGTTCAAGAAGGGAAGTTCCATTGGCCAAATGTTTGAGA

CAACAATGAGAGGAGCGAAGAGAATGGCCATTTTAGGTGACACAGCCTGGGATTTTGGATC
CCTGGGAGGAGTGTTTACATCTATAGGAAAGGCTCTCCACCAAGTTTTCGGAGCAATCTAT
GGGGCTGCTTTTAGTGGGGTCTCATGGACTATGAAAATCCTCATAGGAGTTATTATCACAT
GGATAGGAATGAATTCACGTAGCACCTCACTGTCTGTGTCACTAGTATTGGTGGGAGTCGT
GACACTGTACTTGGGGGTTATGGTGCAGGCT

>PR/DB023 prME nucleotide sequence (SEQ ID NO: 6)

ttccatttaaccacacgtaatggagaaccacacatgatcgttggtaggcaagagaaaggga
aaagtcttctgttcaaaacagaggatggtgttaacatgtgtaccctcatggccatagacct
tggtgaattgtgtgaagatacaatcacgtacaagtgccccctcctcaggcaaaatgaacca
gaagacatagattgttggtgcaactctacgtccacatgggtaacttatgggacatgtacca
ccacaggagaacacagaagagaaaaaagatcagtggcactcgttccacatgtgggcatggg
actggagacacgaactgaaacatggatgtcatcagaaggggcctggaaacatgttcagaga
attgaaacctggatattgagacatccaggctttaccataatggcagcaatcctggcatata
ccataggaacgacacatttccaaagggctctgatcttcattttactgacagccgtcgctcc
ttcaatgacaATGCGTTGCATAGGAATATCAAATAGAGACTTCGTAGAAGGGGTTTCAGGA
GGAAGTTGGGTTGACATAGTCTTAGAACATGGAAGTTGTGTGACGACGATGGCAAAAAATA
AACCAACATTGGATTTTGAACTGATAAAAACAGAAGCCAAACAACCTGCCACTCTAAGGAA
GTACTGTATAGAAGCAAAGCTGACCAATACAACAACAGAATCTCGTTGCCCAACACAAGGG
GAACCCAGTCTAAATGAAGAGCAGGACAAAAGGTTCATCTGCAAACACTCCATGGTAGACA
GAGGATGGGGAAATGGATGTGGATTATTTGGAAAAGGAGGCATTGTAACCTGTGCTATGTT
CACATGCAAAAAGAACATGGAAGGAAAAGTTGTGCTGCCAGAAAATCTGGAATACACCATC
GTGATAACACCTCACTCAGGAGAAGAGCACGCTGTAGGTAATGACACAGGAAAACATGGCA
AGGAAATTAAAATAACACCACAGAGTTCCATCACAGAAGCAGAACTGACAGGCTATGGCAC
TGTCACGATGGAGTGCTCTCCGAGAACGGGCCTCGACTTCAATGAGATGGTGCTGCTGCAG
ATGGAAGACAAAGCCTGGCTGGTGCACAGGCAATGGTTCCTAGATCTGCCGTTACCATGGC
TACCCGGAGCGGACACACAAGGATCAAATTGGATACAGAAAGAGACGTTGGTCACTTTCAA
AAATCCCCACGCGAAGAAACAGGACGTCGTTGTTTTAGGATCTCAAGAAGGGGCCATGCAC
ACGGCACTTACAGGGGCCACAGAAATCCAGATGTCATCAGGAAACTTACTGTTCACAGGAC
ATCTCAAGTGTAGGCTGAGAATGGACAAATTACAGCTTAAAGGAATGTCATACTCTATGTG
TACAGGAAAGTTTAAAATTGTGAAGGAAATAGCAGAAACACAACATGGAACAATAGTTATC
AGAGTACAATATGAAGGGGACGGCTCTCCATGTAAGATTCCTTTTGAGATAATGGATTTAG
AAAAAAGACACGTCCTAGGTCGCCTGATTACAGTGAACCCAATCGTAACAGAAAAAGATAG
CCCAGTCAACATAGAAGCAGAACCTCCATTCGGAGACAGCTACATCATCATAGGAGTAGAG
CCGGGACAATTGAAACTCAATTGGTTCAAGAAGGGAAGTTCCATTGGCCAAATGTTTGAGA
CAACAATGAGAGGAGCGAAGAGAATGGCCATTTTAGGTGACACAGCCTGGGATTTTGGATC
CCTGGGAGGAGTGTTTACATCTATAGGAAAGGCTCTCCACCAAGTTTTCGGAGCAATCTAT
GGGGCTGCTTTTAGTGGGGTCTCATGGACTATGAAAATCCTCATAGGAGTTATCATCACAT
GGATAGGAATGAATTCACGTAGCACCTCACTGTCTGTGTCACTAGTATTGGTGGGAGTCGT
GACACTGTACTTGGGGGTTATGGTGCAGGCT

>MD1280 prME nucleotide sequence (SEQ ID NO: 7)

ttccatttaaccacacgaaatggagaaccacacatgatcgttggcagacaagagaaaggga
aaagccttctgtttaaaacagaggatggtgtgaacatgtgtaccctcatggccattgatct
tggtgaattgtgtgaagatacaatcacgtacaagtgccccctcctcaggcagaatgaacca
gaagatatagattgttggtgcaactccacgtccacatgggtaacttatgggacgtgtacca
ccacaggagaacacagaagagaaaaaagatcagtggcactcgttccacatgtgggtatggg
actggagacacgaactgaaacatggatgtcgtcagaaggggcctggaaacacgctcagaga

attgaaacttggatcttgagacatccaggctttaccataatggcagcaatcctggcatata
ccgtaggaacgacacatttccaaagggccctgattttcatcttactggcagctgtcgctcc
ttcaatgacaATGCGTTGCATAGGAATATCAAATAGAGACTTTGTAGAAGGGGTTTCAGGA
GGAAGCTGGGTTGACATAGTCTTAGAACATGGAAGTTGTGTGACGACAATGGCAAAAAATA
AACCAACACTGGATTTTGAACTGATAAAAACAGAAGCCAAACAACCTGCCACTCTAAGGAA
GTACTGTATAGAGGCAAAGCTGACCAATACAACAACAGAATCTCGTTGCCCAACACAAGGG
GAACCCAGTCTAAATGAAGAGCAGGACAAAAGGTTCGTCTGCAAACACTCCATGGTAGACA
GAGGATGGGGAAATGGATGTGGATTATTTGGAAAGGGAGGCATTGTGACCTGTGCTATGTT
CACATGCAAAAAGAACATGGAAGGAAAAATCGTGCAACCAGAAAATTTGGAATACACCATC
GTGATAACACCTCACTCAGGAGAAGAGCACGCTGTAGGTAATGACACAGGAAAACATGGTA
AGGAAATTAAAATAACACCACAGAGTTCCATCACAGAAGCAGAACTGACAGGCTATGGCAC
AGTCACGATGGAGTGCTCTCCGAGAACGGGCCTTGACTTCAATGAGATGGTGCTGCTGCAG
ATGGAAGATAAAGCTTGGCTGGTGCACAGGCAATGGTTCCTAGACCTGCCGTTACCATGGC
TACCCGGAGCGGACACACAAGGATCAAATTGGATACAGAAAGAGACATTGGTCACTTTCAA
AAATCCCCACGCGAAGAAGCAGGATGTCGTTGTTTTAGGATCTCAAGAAGGAGCCATGCAC
ACGGCACTCACAGGGGCCACAGAAATCCAGATGTCATCAGGAAACTTACTATTCACAGGAC
ATCTCAAATGCAGGCTGAGAATGGACAAACTACAGCTCAAAGGAATGTCATACTCTATGTG
TACAGGAAAGTTTAAAATTGTGAAGGAAATAGCAGAAACACAACATGGAACAATAGTTATC
AGAGTACAATATGAAGGAGACGGCTCTCCATGTAAGATCCCTTTTGAAATAATGGATTTGG
AAAAAAGACATGTCTTAGGTCGCCTGATTACAGTTAATCCGATCGTAACAGAAAAAGATAG
CCCAGTCAACATAGAAGCAGAACCTCCATTCGGAGACAGCTACATCATTATAGGAGTAGAG
CCGGGACAATTGAAACTCAACTGGTTCAAGAAAGGAAGTTCCATCGGCCAAATGTTTGAGA
CGACAATGAGAGGAGCAAAGAGAATGGCCATTTTAGGTGACACAGCCTGGGATTTTGGATC
TCTGGGAGGAGTGTTTACATCTATAGGAAAGGCTCTCCACCAAGTTTTCGGAGCAATCTAT
GGGGCTGCCTTTAGTGGGGTTTCATGGACTATGAAAATCCTCATAGGAGTCATCATCACAT
GGATAGGAATGAATTCACGTAGCACCTCACTGTCTGTGTCACTAGTATTGGTGGGAATCAT
AACACTGTACTTGGGAGCTATGGTGCAGGCT

Corresponding protein prM to E sequences of the four selected strains

>LAV2 prME protein sequence (SEQ ID NO: 8)

fhlttrngephmivsrqekgksllfktevgvnmctlmamdlgelcedtitykcpllrqnep
edidcwcnststwvtygtcttmgehrrekrsvalvphvgmgletrtetwmssegawkhvqr
ietwilrhpgftmmaailaytigtthfqralifilltavtpsmtMRCIGMSNRDFVEGVSG
GSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTESRCPTQG
EPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFRCKKNMEGKVVQPENLEYTI
VITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQ
MENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMH
TALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGKFKVVKEIAETQHGTIVI
RVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYIIIGVE
PGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIY
GAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVTLVLVGIVTLYLGVMVQA

>LAV2 E protein sequence (SEQ ID NO: 13)

MRCIGMSNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCI
EAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFRCK
KNMEGKVVQPENLEYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTM
ECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPH

AKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGK
FKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGG
VFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVTLVLVGIVTLY
LGVMVQA

>LAV2 M protein sequence (SEQ ID NO: 19)

svalvphvgmgletrtetwmssegawkhvqrietwilrhpgftmmaailaytigtthfqra
lifilltavtpsmt

>BID/V585 prME protein sequence (SEQ ID NO: 9)

fhlttrngephmivgrqekgksllfktedgvnmctlmaidlgelcedtitykcpllrqnep
edidcwcnststwvtygtctttgehrrekrsvalvphvgmgletrtetwmssegawkhvqr
ietwilrhpgftimaailaytigtthfqralifilltavapsmtMRCIGISNRDFVEGVSG
GSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTESRCPTQG
EPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKVVQPENLEYTI
VITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQ
MEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMH
TALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGKFKIVKEIAETQHGTIVI
RVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYIIIGVE
PGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIY
GAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA

>BID/V585 E protein sequence (SEQ ID NO: 14)

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCI
EAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAMFTCK
KNMEGKVVQPENLEYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTM
ECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPH
AKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGK
FKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGG
VFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLY
LGVMVQA

>BID/V585 M protein sequence (SEQ ID NO: 20)

svalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtthfqra
lifilltavapsmt

>PR/DB023 prME protein sequence (SEQ ID NO: 10)

fhlttrngephmivgrqekgksllfktedgvnmctlmaidlgelcedtitykcpllrqnep
edidcwcnststwvtygtctttgehrrekrsvalvphvgmgletrtetwmssegawkhvqr
ietwilrhpgftimaailaytigtthfqralifilltavapsmtMRCIGISNRDFVEGVSG
GSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTESRCPTQG
EPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKVVLPENLEYTI
VITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQ
MEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMH

TALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGKFKIVKEIAETQHGTIVI
RVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYIIIGVE
PGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIY
GAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA

>PR/DB023 E protein sequence (SEQ ID NO: 15)

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCI
EAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAMFTCK
KNMEGKVVLPENLEYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTM
ECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPH
AKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGK
FKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGG
VFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLY
LGVMVQA

>PR/DB023 M protein sequence (SEQ ID NO : 21)

svalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtthfqra
lifilltavapsmt

>MD1280 prME protein sequence (SEQ ID NO: 11)

fhlttrngephmivgrqekgksllfktedgvnmctlmaidlgelcedtitykcpllrqnep
edidcwcnststwvtygtctttgehrrekrsvalvphvgmgletrtetwmssegawkhaqr
ietwilrhpgftimaailaytvgtthfqralifillaavapsmtMRCIGISNRDFVEGVSG
GSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTESRCPTQG
EPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENLEYTI
VITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQ
MEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMH
TALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGKFKIVKEIAETQHGTIVI
RVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYIIIGVE
PGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIY
GAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGIITLYLGAMVQA

>MD1280 E protein sequence (SEQ ID NO: 16)

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCI
EAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCK
KNMEGKIVQPENLEYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTM
ECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPH
AKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGK
FKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGG
VFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGIITLY
LGAMVQA

>MD1280 M protein sequence (SEQ ID NO: 22)

svalvphvgmgletrtetwmssegawkhaqrietwilrhpgftimaailaytvgtthfqra
lifillaavapsmt

>Consensus M sequence (SEQ ID NO: 17)

svalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtthfqra
lifilltavapsmt

Example 3: Construction of the cDNA clones corresponding to the optimized serotype 2 chimeric viruses and production of the encoded viruses

Construction of chimeric dengue viruses corresponding to the optimized serotype 2 strains is achieved using the Chimerivax™ technology substantially in accordance with the teaching of Chambers, et al. (1999, J. Virology 73(4):3095-3101). Reference may also be made to international patent applications WO 98/37911, WO 03/101397, WO 07/021672, WO 08/007021, WO 08/047023 and WO 08/065315, which detail the analogous processes used to construct CYD-1, CYD2, CYD-3 and CYD-4. Briefly, however, chimeric dengue viruses corresponding to the optimized serotype 2 strains are constructed as follows (N.B. the optimized chimeric dengue viruses are constructed using the genomic backbone of YF strain YF17D204 (YF-VAX(R), Sanofi-Pasteur, Swiftwater, PA, USA).

Construction of plasmid pSP1101

Construction of the YF-VAX cDNA clone - pJSY2284.1 (pACYC YF-Vax 5-3)

A full-length infectious cDNA clone of YF-VAX is constructed. The full-length infectious cDNA clone is based on the sequence of YF-VAX. A low copy number plasmid pACYC177 (New England Biolabs, Inc., Ipswich, MA, USA) is used to assemble the full-length cDNA clone.

A DNA sequence named as SP6 YF-Vax 5-3 is synthesized by GeneArt®. The sequence of SP6 YF-Vax 5-3 is designed in a way to facilitate an easy assembly of a full-length YF-Vax cDNA clone. The sequence is 2897 bp long and comprises the Xma I-SP6 promoter, the YF-Vax 5'UTR, the capsid, prM, M, part of E which extends to the Apa I site followed by unique sites Mlu I-Sap I-Ngo MI-Aat II-Cla I for assembly, part of NS5 and further extended to 3' UTR followed by an Nru I site, which is used for run-off. This synthesized DNA sequence is flanked by EcoR V and Xho I sites. After digestion with EcoR V/Xho I, this DNA fragment is then cloned into the Aat II/Xho I sites of low copy number plasmid pACYC177 to replace the 1615bp Aat II/Xho I fragment. The resulting plasmid pJSY2284.1 (pACYC YF-Vax 5-3) is confirmed by sequence analysis.

RT-PCR and cloning of the YF-Vax cDNA fragments spanning from the sites Apa I, Mlu I, Sap I, Ngo MI, Aat II and Cla I and assembly of a full-length infectious cDNA clone of YF-vax (pJSY2374.5)

The yellow fever vaccine YF-VAX is grown in Vero cells, and the virus particles are concentrated. The viral RNA of YF-VAX is extracted from the concentrated virus and the cDNA copy is made by reverse transcriptase. Five cDNA fragments as shown herein are PCR amplified, TOPO cloned, sequenced and compared to the sequence of YF-VAX 2003. The PCR errors found in each fragment are corrected by either site-directed mutagenesis or fragment switching. There are too many sequence differences found in Ngo MI-Aat II fragment after TOPO cloning, and therefore, this fragment is synthesized by GeneArt®. After final sequence confirmation, the five DNA fragments; Apa I-Mlu I, Mlu I-Sap I, Sap I-Ngo M1, Ngo MI-Aat II, and Aat II-Cla I are isolated and stepwise cloned into the unique sites Apa I, Mlu I, Sap I, Ngo MI, Aat II and Cla I in the plasmid pJSY2284.1 to obtain plasmid pJSY2374.5, which is confirmed to contain the correct sequence of YF-VAX full-length cDNA.

Construction of cDNA for optimized chimeric dengue virus derived from the LAV2 strain (pSP1101)

The strategy is to replace the prM and E genes of the YF-VAX® vaccine strain in the pJSY2374.5 plasmid containing the YF-VAX genome with those of the LAV2 strain, as done previously to build the CYD-1, CYD-2, CYD-3 and CYD-4 dengue vaccines, using the Chimerivax™ technology. The resulting plasmid is pSP1101.

In pJSY2374, restriction sites used for cloning are Xma I and Mlu I. These sites are located upstream and downstream of a 3000 bp fragment which contains: the SP6 promoter, YF17D 5'UTR, YF17D-capsid, YF17D-prM, YF17D-E and the N terminus of YF17D-NS1. A sequence corresponding to this fragment but instead containing the prM and E genes of LAV2 flanked by Xma I and Mlu I sites is synthesized by GeneArt® and cloned into plasmid pMK-RQ (GeneArt®, Life Technologies Ltd, Paisley, U.K.) to create plasmid pMK-RQ-Seq1. Plasmid pJSY2374.5 and pMK-RQ-Seq1 are digested by Xma I and Mlu 1. The Xma I-Mlu I fragment from pMK-RQ-Seq1 is then inserted into plasmid pJSY2374.5 to form plasmid pSP1101. XL-10 Gold Ultracompetent bacteria (Agilent Technologies, CA, USA) are used for transformation, as they are suitable for large plasmids. In a second step, positive clones are transferred into One Shot® TOP10 *E. coli* (Life Technologies Ltd, Paisley, U.K.), which allows the amplification of large size plasmids in significant amounts.

Plasmid pSP1101 thus allows the expression of LAV2 strain prM and E proteins with a YF-VAX replication engine. The resulting chimeric virus is designated CYD-LAV. Sequencing analysis shows no mutation as compared to the original sequences.

Construction of corresponding plasmids for strains BID-V585, PR/DB023 and MD1280

An analogous strategy to that described above is used to build the plasmids corresponding to the serotype 2 strains BID-V585, PR/DB023 and MD1280. These plasmids are designated pSP1102 (BID-V585), pSP1103 (PR/DB023) and pSP1104 (MD1280). The resulting chimeric viruses generated from those plasmids are designated CYD-BID, CYD-PR and CYD-MD. Sequence analysis of the generated plasmids shows no mutations compared to the original sequences.

Generation of chimeric viruses from plasmids pSP1101, pSP1102, pSP1103 and pSP1104

In vitro transcription of RNA and generation of viruses is carried out as previously described (Guirakhoo F et al. J. Virol. 2001; 75:7290-304).

Example 4: Evaluation of the immunogenicity and viremia of the optimized serotype 2 chimeric viruses in a monkey model

Evaluation of immunogenicity and viremia in monkeys

Design of the study

Four groups each containing four Cynomolgus monkeys are defined. The four groups receive the following formulations (containing 5 $\log_{10}$ $CCID_{50}$ of each CYD dengue serotype):

1. Control tetravalent formulation, i.e. a formulation comprising CYD-1, CYD-2, CYD-3 and CYD-4.
2. CYD-LAV tetravalent formulation, i.e. a formulation comprising CYD-1, CYD-3, CYD-4 and CYD-LAV.
3. CYD-MD tetravalent formulation, i.e. a formulation comprising CYD-1, CYD-3, CYD-4 and CYD-MD.
4. CYD-PR tetravalent formulation, i.e. a formulation comprising CYD-1, CYD-3, CYD-4 and CYD-PR.

Monkeys receive two doses two months apart, as previously described (Guy B et al., Am J Trop Med Hyg. 2009; 80(2):302-11).

Results

Immunogenicity (SN_{50} neutralizing response) and viremia are determined as described in the Materials and Methods section of Guy B., et al., Am. J. Trop. Med. Hyg. 2009; 80(2): 302-11.

Table 6: SN_{50} neutralizing responses in monkeys immunized with optimized chimeric dengue serotype 2 viruses

		PD1				PD2			
		DEN1	DEN2	DEN3	DEN4	DEN1	DEN2	DEN3	DEN4
control CYD TV	responders	4/4	2/4	1/4	4/4	4/4	1/4	4/4	4/4
	GMT	**27**	**5**	**7**	**636**	**71**	**8**	**35**	**425**
CYD-LAV TV	responders	4/4	2/4	4/4	4/4	4/4	4/4	4/4	4/4
	GMT	**95**	**63**	**19**	**477**	**189**	**95**	**80**	**477**
CYD-MD TV	responders	4/4	4/4	3/4	4/4	4/4	4/4	4/4	4/4
	GMT	**33**	**100**	**8**	**63**	**35**	**63**	**16**	**100**
CYD-PR TV	responders	3/4	4/4	1/4	4/4	4/4	4/4	4/4	4/4
	GMT	**27**	**63**	**7**	**168**	**109**	**84**	**38**	**212**

PD: Post-dose; TV: tetravalent formulation

No serotype 2 viremia is observed, regardless of the serotype 2 chimeric virus administered. In respect of immunogenicity responses against DEN2, the tetravalent formulations comprising CYD-LAV, CYD-MD and CYD-PR demonstrate a higher response (both GMTs and number of responding animals) than the control formulation (see Table 6).

Example 5. Assessment of tetravalent dengue vaccine formulations in flavivirus-naïve adults in Mexico.

The objective of the present study was to compare the immunogenicity and viremia of a blended tetravalent dengue vaccine comprising CYD-1 (i.e. the particular Chimerivax dengue serotype 1 (CYD-1) strain generated from the prM and E sequences of DEN1 PU0359 (TYP 1 140)), VDV2, CYD-3 (i.e. the particular Chimerivax dengue serotype 3 (CYD-3) strain generated from the prM and E sequences of DEN3 PaH881/88) and CYD-4 (i.e. the particular Chimerivax dengue serotype 4 (CYD-4) strain generated from the prM and E sequences of DEN4 1228 (TVP 980)) with the immunogenicity and viremia of a tetravalent dengue vaccine comprising CYD-1, CYD-2 (i.e. the particular Chimerivax dengue serotype 2 (CYD-2) strain generated from the prM and E sequences of DEN2 PUO218), CYD-3 and CYD-4. See Example 1 for more detail concerning the particular CYD-1, CYD-2, CYD-3 and CYD-4 used in this study.

The relevant nucleotide and protein sequences of the VDV2 strain are as follows:

>VDV2 nucleotide sequence (SEQ ID NO: 24)

AGUUGUUAGUCUACGUGGACCGACAAAGACAGAUUCUUUGAGGGAGCUAAGCUCAAUGUAG
UUCUAACAGUUUUUUAAUUAGAGAGCAGAUCUCUGAUGAAUAACCAACGGAAAAAGGCGAA

AAACACGCCUUUCAAUAUGCUGAAACGCGAGAGAAACCGCGUGUCGACUGUGCAACAGCUG
ACAAAGAGAUUCUCACUUGGAAUGCUGCAGGGACGAGGACCAUUAAAACUGUUCAUGGCCC
UGGUGGCGUUCCUUCGUUUCCUAACAAUCCCACCAACAGCAGGGAUAUUGAAGAGAUGGGG
AACAAUUAAAAAAUCAAAAGCUAUUAAUGUUUUGAGAGGGUUCAGGAAAGAGAUUGGAAGG
AUGCUGAACAUCUUGAAUAGGAGACGCAGAUCUGCAGGCAUGAUCAUUAUGCUGAUUCCAA
CAGUGAUGGCGUUCCAUUUAACCACACGUAACGGAGAACCACACAUGAUCGUCAGCAGACA
AGAGAAAGGGAAAAGUCUUCUGUUUAAAACAGAGGUUGGCGUGAACAUGUGUACCCUCAUG
GCCAUGGACCUUGGUGAAUUGUGUGAAGACACAAUCACGUACAAGUGUCCCCUUCUCAGGC
AGAAUGAGCCAGAAGACAUAGACUGUUGGUGCAACUCUACGUCCACGUGGGUAACUUAUGG
GACGUGUACCACCAUGGGAGAACAUAGAAGAGAAAAAAGAUCAGUGGCACUCGUUCCACAU
GUGCGAAUGGGACUGGAGACACGAACUGAAACAUGGAUGUCAUCAGAAGGGGCCUGGAAAC
AUGUCCAGAGAAUUGAAACUUGGAUCUUGAGACAUCCAGGCUUCACCAUGAUGGCAGCAAU
CCUGGCAUACACCAUAGGAACGACACAUUUCCAAAGAGCCCUGAUUUUCAUCUUACUGACA
GCUGUCACUCCUUCAAUGACAAUGCGUUGCAUAGGAAUGUCAAAUAGAGACUUUGUGGAAG
GGGUUUCAGGAGGAAGCUGGGUUGACAUAGUCUUAGAACAUGGAAGCUGUGUGACGACGAU
GGCAAAAAACAAACCAACAUUGGAUUUUGAACUGAUAAAAACAGAAGCCAAACAGCCUGCC
ACCCUAAGGAAGUACUGUAUAGAGGCAAAGCUAACCAACACAACAACAGAAUCUCGCUGCC
CAACACAAGGGGAACCCAGCCUAAAUGAAGAGCAGGACAAAAGGUUCGUCUGCAAACACUC
CAUGGUAGACAGAGGAUGGGGAAAUGGAUGUGGACUAUUUGGAAAGGGAGGCAUUGUGACC
UGUGCUAUGUUCAGAUGCAAAAAGAACAUGGAAGGAAAAGUUGUGCAACCAGAAAACUUGG
AAUACACCAUUGUGAUAACACCUCACUCAGGGGAAGAGCAUGCAGUCGGAAAUGACACAGG
AAAACAUGGCAAGGAAAUCAAAAUAACACCACAGAGUUCCAUCACAGAAGCAGAAUUGACA
GGUUAUGGCACUGUCACAAUGGAGUGCUCUCCAAGAACGGGCCUCGACUUCAAUGAGAUGG
UGUUGCUGCAGAUGGAAAAUAAAGCUUGGCUGGUGCACAGGCAAUGGUUCCUAGACCUGCC
GUUACCAUGGUUGCCCGGAGCGGACACACAAGAGUCAAAUUGGAUACAGAAGGAGACAUUG
GUCACUUUCAAAAAUCCCCAUGCGAAGAAACAGGAUGUUGUUGUUUUAGGAUCCCAAGAAG
GGGCCAUGCACACAGCACUUACAGGGGCCACAGAAAUCCAAAUGUCAUCAGGAAACUUACU
CUUCACAGGACAUCUCAAGUGCAGGCUGAGAAUGGACAAGCUACAGCUCAAAGGAAUGUCA
UACUCUAUGUGCACAGGAAAGUUUAAAGUUGUGAAGGAAAUAGCAGAAACACAACAUGGAA
CAAUAGUUAUCAGAGUGCAAUAUGAAGGGGACGGCUCUCCAUGCAAGAUCCCUUUUGAGAU
AAUGGAUUUGGAAAAAAGACAUGUCUUAGGUCGCCUGAUUACAGUCAACCCAAUUGUGACA
GAAAAAGAUAGCCCAGUCAACAUAGAAGCAGAACCUCCAUUUGGAGACAGCUACAUCAUCA
UAGGAGUAGAGCCGGGACAACUGAAGCUCAACUGGUUUAAGAAAGGAAGUUCUAUCGGCCA
AAUGUUUGAGACAACAAUGAGGGGGGCGAAGAGAAUGGCCAUUUUAGGUGACACAGCCUGG
GAUUUUGGAUCCUUGGGAGGAGUGUUUACAUCUAUAGGAAAGGCUCUCCACCAAGUCUUUG
GAGCAAUCUAUGGAGCUGCCUUCAGUGGGGUUUCAUGGACUAUGAAAAUCCUCAUAGGAGU
CAUUAUCACAUGGAUAGGAAUGAAUUCACGCAGCACCUCACUGUCUGUGACACUAGUAUUG
GUGGGAAUUGUGACACUGUAUUUGGGAGUCAUGGUGCAGGCCGAUAGUGGUUGCGUUGUGA
GCUGGAAAAACAAAGAACUGAAAUGUGGCAGUGGGAUUUUCAUCACAGACAACGUGCACAC
AUGGACAGAACAAUACAAAUUCCAACCAGAAUCCCCUUCAAAACUAGCUUCAGCUAUCCAG
AAAGCCCAUGAAGAGGACAUUUGUGGAAUCCGCUCAGUAACAAGACUGGAGAAUCUGAUGU
GGAAACAAAUAACACCAGAAUUGAAUCACAUUCUAUCAGAAAAUGAGGUGAAGUUAACUAU
UAUGACAGGAGACAUCAAAGGAAUCAUGCAGGCAGGAAAACGAUCUCUGCGGCCUCAGCCC
ACUGAGCUGAAGUAUUCAUGGAAAACAUGGGGCAAAGCAAAAAUGCUCUCUACAGAGUCUC
AUAACCAGACCUUUCUCAUUGAUGGCCCCGAAACAGCAGAAUGCCCCAACACAAAUAGAGC
UUGGAAUUCGUUGGAAGUUGAAGACUAUGGCUUUGGAGUAUUCACCACCAAUAUAUGGCUA
AAAUUGAAAGAAAAACAGGAUGUAUUCUGCGACUCAAAACUCAUGUCAGCGGCCAUAAAAG
ACAACAGAGCCGUCCAUGCCGAUAUGGGUUAUUGGAUAGAAAGUGCACUCAAUGACACAUG
GAAGAUAGAGAAAGCCUCUUUCAUUGAAGUUAAAAACUGCCACUGGCCAAAAUCACACACC
CUCUGGAGCAAUGGAGUGCUAGAAAGUGAGAUGAUAAUUCCAAAGAAUCUCGCUGGACCAG

UGUCUCAACACAACUAUAGACCAGGCUACCAUACACAAAUAACAGGACCAUGGCAUCUAGG
UAAGCUUGAGAUGGACUUUGAUUUCUGUGAUGGAACAACAGUGGUAGUGACUGAGGACUGC
GGAAAUAGAGGACCCUCUUUGAGAACAACCACUGCCUCUGGAAAACUCAUAACAGAAUGGU
GCUGCCGAUCUUGCACAUUACCACCGCUAAGAUACAGAGGUGAGGAUGGGUGCUGGUACGG
GAUGGAAAUCAGACCAUUGAAGGAGAAAGAAGAGAAUUUGGUCAACUCCUUGGUCACAGCU
GGACAUGGGCAGGUCGACAACUUUUCACUAGGAGUCUUGGGAAUGGCAUUGUUCCUGGAGG
AAAUGCUUAGGACCCGAGUAGGAACGAAACAUGCAAUACUACUAGUUGCAGUUUCUUUUGU
GACAUUGAUCACAGGGAACAUGUCCUUUAGAGACCUGGGAAGAGUGAUGGUUAUGGUAGGC
GCCACUAUGACGGAUGACAUAGGUAUGGGCGUGACUUAUCUUGCCCUACUAGCAGCCUUCA
AAGUCAGACCAACUUUUGCAGCUGGACUACUCUUGAGAAAGCUGACCUCCAAGGAAUUGAU
GAUGACUACUAUAGGAAUUGUACUCCUCUCCCAGAGCACCAUACCAGAGACCAUUCUUGAG
UUGACUGAUGCGUUAGCCUUAGGCAUGAUGGUCCUCAAAAUGGUGAGAAAUAUGGAAAAGU
AUCAAUUGGCAGUGACUAUCAUGGCUAUCUUGUGCGUCCCAAACGCAGUGAUAUUACAAAA
CGCAUGGAAAGUGAGUUGCACAAUAUUGGCAGUGGUGUCCGUUUCCCCACUGUUCUUAACA
UCCUCACAGCAAAAAACAGAUUGGAUACCAUUAGCAUUGACGAUCAAAGGUCUCAAUCCAA
CAGCUAUUUUUCUAACAACCCUCUCAAGAACCAGCAAGAAAAGGAGCUGGCCAUUAAAUGA
GGCUAUCAUGGCAGUCGGGAUGGUGAGCAUUUUAGCCAGUUCUCUCCUAAAAAAUGAUAUU
CCCAUGACAGGACCAUUAGUGGCUGGAGGGCUCCUCACUGUGUGCUACGUGCUCACUGGAC
GAUCGGCCGAUUUGGAACUGGAGAGAGCAGCCGAUGUCAAAUGGGAAGACCAGGCAGAGAU
AUCAGGAAGCAGUCCAAUCCUGUCAAUAACAAUAUCAGAAGAUGGUAGCAUGUCGAUAAAA
AAUGAAGAGGAAGAACAAACACUGACCAUACUCAUUAGAACAGGAUUGCUGGUGAUCUCAG
GACUUUUUCCUGUAUCAAUACCAAUCACGGCAGCAGCAUGGUACCUGUGGGAAGUGAAGAA
ACAACGGGCCGGAGUAUUGUGGGAUGUUCCUUCACCCCCACCCAUGGGAAAGGCUGAACUG
GAAGAUGGAGCCUAUAGAAUUAAGCAAAAAGGGAUUCUUGGAUAUUCCCAGAUCGGAGCCG
GAGUUUACAAAGAAGGAACAUUCCAUACAAUGUGGCAUGUCACACGUGGCGCUGUUCUAAU
GCAUAAAGGAAAGAGGAUUGAACCAACAUGGGCGGACGUCAAGAAAGACCUAAUAUCAUAU
GGAGGAGGCUGGAAGUUAGAAGGAGAAUGGAAGGAAGGAGAAGAAGUCCAGGUAUUGGCAC
UGGAGCCUGGAAAAAAUCCAAGAGCCGUCCAAACGAAACCUGGUCUUUUCAAAACCAACGC
CGGAACAAUAGGUGCUGUAUCUCUGGACUUUUCUCCUGGAACGUCAGGAUCUCCAAUUAUC
GACAAAAAAGGAAAAGUUGUGGGUCUUUAUGGUAAUGGUGUUGUUACAAGGAGUGGAGCAU
AUGUGAGUGCUAUAGCCCAGACUGAAAAAAGCAUUGAAGACAACCCAGAGAUCGAAGAUCA
CAUUUUCCGAAAGAGAAGACUGACCAUCAUGGACCUCCACCCAGGAGCGGGAAAGACGAAG
AGAUACCUUCCGGCCAUAGUCAGAGAAGCUAUAAAACGGGGUUUGAGAACAUUAAUCUUGG
CCCCCACUAGAGUUGUGGCAGCUGAAAUGGAGGAAGCCCUUAGAGGACUUCCAAUAAGAUA
CCAGACCCCAGCCAUCAGAGCUGAGCACACCGGGCGGGAGAUUGUGGACCUAAUGUGUCAU
GCCACAUUUACCAUGAGGCUGCUAUCACCAGUUAGAGUGCCAAACUACAACCUGAUUAUCA
UGGACGAAGCCCAUUUCACAGACCCAGCAAGUAUAGCAGCUAGAGGAUACAUCUCAACUCG
AGUGGAGAUGGGUGAGGCAGCUGGGAUUUUUAUGACAGCCACUCCCCCGGGAAGCAGAGAC
CCAUUUCCUCAGAGCAAUGCACCAAUCAUAGAUGAAGAAAGAGAAAUCCCUGAACGCUCGU
GGAAUUCCGGACAUGAAUGGGUCACGGAUUUUAAAGGGAAGACUGUUUGGUUCGUUCCAAG
UAUAAAAGCAGGAAAUGAUAUAGCAGCUUGCCUGAGGAAAAAUGGAAAGAAAGUGAUACAA
CUCAGUAGGAAGACCUUUGAUUCUGAGUAUGUCAAGACUAGAACCAAUGAUUGGGACUUCG
UGGUUACAACUGACAUUUCAGAAAUGGGUGCCAAUUUCAAGGCUGAGAGGGUUAUAGACCC
CAGACGCUGCAUGAAACCAGUCAUACUAACAGAUGGUGAAGAGCGGGUGAUUCUGGCAGGA
CCUAUGCCAGUGACCCACUCUAGUGCAGCACAAAGAAGAGGGAGAAUAGGAAGAAAUCCAA
AAAAUGAGAAUGACCAGUACAUAUACAUGGGGGAACCUCUGGAAAAUGAUGAAGACUGUGC
ACACUGGAAAGAAGCUAAAAUGCUCCUAGAUAACAUCAACACGCCAGAAGGAAUCAUUCCU
AGCAUGUUCGAACCAGAGCGUGAAAAGGUGGAUGCCAUUGAUGGCGAAUACCGCUUGAGAG
GAGAAGCAAGGAAAACCUUUGUAGACUUAAUGAGAAGAGGAGACCUACCAGUCUGGUUGGC
CUACAGAGUGGCAGCUGAAGGCAUCAACUACGCAGACAGAAGGUGGUGUUUUGAUGGAGUC

AAGAACAACCAAAUCCUAGAAGAAAACGUGGAAGUUGAAAUCUGGACAAAAGAAGGGGAAA
GGAAGAAAUUGAAACCCAGAUGGUUGGAUGCUAGGAUCUAUUCUGACCCACUGGCGCUAAA
AGAAUUUAAGGAAUUUGCAGCCGGAAGAAAGUCUCUGACCCUGAACCUAAUCACAGAAAUG
GGUAGGCUCCCAACCUUCAUGACUCAGAAGGCAAGAGACGCACUGGACAACUUAGCAGUGC
UGCACACGGCUGAGGCAGGUGGAAGGGCGUACAACCAUGCUCUCAGUGAACUGCCGGAGAC
CCUGGAGACAUUGCUUUUACUGACACUUCUGGCUACAGUCACGGGAGGGAUCUUUUUAUUC
UUGAUGAGCGCAAGGGGCAUAGGGAAGAUGACCCUGGGAAUGUGCUGCAUAAUCACGGCUA
GCAUCCUCCUAUGGUACGCACAAAUACAGCCACACUGGAUAGCAGCUUCAAUAAUACUGGA
GUUUUUUCUCAUAGUUUUGCUUAUUCCAGAACCUGAAAAACAGAGAACACCCCAAGACAAC
CAACUGACCUACGUUGUCAUAGCCAUCCUCACAGUGGUGGCCGCAACCAUGGCAAACGAGA
UGGGUUUCCUAGAAAAAACGAAGAAAGAUCUCGGAUUGGGAAGCAUUGCAACCCAGCAACC
CGAGAGCAACAUCCUGGACAUAGAUCUACGUCCUGCAUCAGCAUGGACGCUGUAUGCCGUG
GCCACAACAUUUGUUACACCAAUGUUGAGACAUAGCAUUGAAAAUUCCUCAGUGAAUGUGU
CCCUAACAGCUAUAGCCAACCAAGCCACAGUGUUAAUGGGUCUCGGGAAAGGAUGGCCAUU
GUCAAAGAUGGACAUCGGAGUUCCCCUUCUCGCCAUUGGAUGCUACUCACAAGUCAACCCC
AUAACUCUCACAGCAGCUCUUUUCUUAUUGGUAGCACAUUAUGCCAUCAUAGGGCCAGGAC
UCCAAGCAAAAGCAACCAGAGAAGCUCAGAAAAGAGCAGCGGCGGGCAUCAUGAAAAACCC
AACUGUCGAUGGAAUAACAGUGAUUGACCUAGAUCCAAUACCUUAUGAUCCAAAGUUUGAA
AAGCAGUUGGGACAAGUAAUGCUCCUAGUCCUCUGCGUGACUCAAGUAUUGAUGAUGAGGA
CUACAUGGGCUCUGUGUGAGGCUUUAACCUUAGCUACCGGGCCCAUCUCCACAUUGUGGGA
AGGAAAUCCAGGGAGGUUUUGGAACACUACCAUUGCGGUGUCAAUGGCUAACAUUUUUAGA
GGGAGUUACUUGGCCGGAGCUGGACUUCUCUUUUCUAUUAUGAAGAACACAACCAACACAA
GAAGGGGAACUGGCAACAUAGGAGAGACGCUUGGAGAGAAAUGGAAAAGCCGAUUGAACGC
AUUGGGAAAAAGUGAAUUCCAGAUCUACAAGAAAAGUGGAAUCCAGGAAGUGGAUAGAACC
UUAGCAAAAGAAGGCAUUAAAAGAGGAGAAACGGACCAUCACGCUGUGUCGCGAGGCUCAG
CAAAACUGAGAUGGUUCGUUGAGAGAAACAUGGUCACACCAGAAGGGAAAGUAGUGGACCU
CGGUUGUGGCAGAGGAGGCUGGUCAUACUAUUGUGGAGGACUAAAGAAUGUAAGAGAAGUC
AAAGGCCUAACAAAAGGAGGACCAGGACACGAAGAACCCAUCCCCAUGUCAACAUAUGGGU
GGAAUCUAGUGCGUCUUCAAAGUGGAGUUGACGUUUUCUUCAUCCCGCCAGAAAAGUGUGA
CACAUUAUUGUGUGACAUAGGGGAGUCAUCACCAAAUCCCACAGUGGAAGCAGGACGAACA
CUCAGAGUCCUUAACUUAGUAGAAAAUUGGUUGAACAACAACACUCAAUUUUGCAUAAAGG
UUCUCAACCCAUAUAUGCCCUCAGUCAUAGAAAAAAUGGAAGCACUACAAAGGAAAUAUGG
AGGAGCCUUAGUGAGGAAUCCACUCUCACGAAACUCCACACAUGAGAUGUACUGGGUAUCC
AAUGCUUCCGGGAACAUAGUGUCAUCAGUGAACAUGAUUUCAAGGAUGUUGAUCAACAGAU
UUACAAUGAGAUACAAGAAAGCCACUUACGAGCCGGAUGUUGACCUCGGAAGCGGAACCCG
UAACAUCGGGAUUGAAAGUGAGAUACCAAACCUAGAUAUAAUUGGGAAAAGAAUAGAAAAA
AUAAAGCAAGAGCAUGAAACAUCAUGGCACUAUGACCAAGACCACCCAUACAAAACGUGGG
CAUACCAUGGUAGCUAUGAAACAAAACAGACUGGAUCAGCAUCAUCCAUGGUCAACGGAGU
GGUCAGGCUGCUGACAAAACCUUGGGACGUUGUCCCCAUGGUGACACAGAUGGCAAUGACA
GACACGACUCCAUUUGGACAACAGCGCGUUUUUAAAGAGAAAGUGGACACGAGAACCCAAG
AACCGAAAGAAGGCACGAAGAAACUAAUGAAAAUAACAGCAGAGUGGCUUUGGAAAGAAUU
AGGGAAGAAAAAGACACCCAGGAUGUGCACCAGAGAAGAAUUCACAAGAAAGGUGAGAAGC
AAUGCAGCCUUGGGGGCCAUAUUCACUGAUGAGAACAAGUGGAAGUCGGCACGUGAGGCUG
UUGAAGAUAGUAGGUUUUGGGAGCUGGUUGACAAGGAAAGGAAUCUCCAUCUUGAAGGAAA
GUGUGAAACAUGUGUGUACAACAUGAUGGGAAAAAGAGAGAAGAAGCUAGGGGAAUUCGGC
AAGGCAAAAGGCAGCAGAGCCAUAUGGUACAUGUGGCUUGGAGCACGCUUCUUAGAGUUUG
AAGCCCUAGGAUUCUUAAAUGAAGAUCACUGGUUCUCCAGAGAGAACUCCCUGAGUGGAGU
GGAAGGAGAAGGGCUGCACAAGCUAGGUUACAUUCUAAGAGACGUGAGCAAGAAAGAGGGA
GGAGCAAUGUAUGCCGAUGACACCGCAGGAUGGGAUACAAAAAUCACACUAGAAGACCUAA
AAAAUGAAGAGAUGGUAACAAACCACAUGGAAGGAGAACACAAGAAACUAGCCGAGGCCAU

UUUCAAACUAACGUACCAAAACAAGGUGGUGCGUGUGCAAAGACCAACACCAAGAGGCACA
GUAAUGGACAUCAUAUCGAGAAGAGACCAAAGAGGUAGUGGACAAGUUGGCACCUAUGGAC
UCAAUACUUUCACCAAUAUGGAAGCCCAACUAAUCAGACAGAUGGAGGGAGAAGGAGUCUU
UAAAAGCAUUCAGCACCUAACAAUCACAGAAGAAAUCGCUGUGCAAAACUGGUUAGCAAGA
GUGGGGCGCGAAAGGUUAUCAAGAAUGGCCAUCAGUGGAGAUGAUUGUGUUGUGAAACCUU
UAGAUGACAGGUUCGCAAGCGCUUUAACAGCUCUAAAUGACAUGGGAAAGAUUAGGAAAGA
CAUACAACAAUGGGAACCUUCAAGAGGAUGGAAUGAUUGGACACAAGUGCCCUUCUGUUCA
CACCAUUUCCAUGAGUUAAUCAUGAAAGACGGUCGCGUACUCGUUGUUCCAUGUAGAAACC
AAGAUGAACUGAUUGGCAGAGCCCGAAUCUCCCAAGGAGCAGGGUGGUCUUUGCGGGAGAC
GGCCUGUUUGGGGAAGUCUUACGCCCAAAUGUGGAGCUUGAUGUACUUCCACAGACGCGAC
CUCAGGCUGGCGGCAAAUGCUAUUUGCUCGGCAGUACCAUCACAUUGGGUUCCAACAAGUC
GAACAACCUGGUCCAUACAUGCUAAACAUGAAUGGAUGACAACGGAAGACAUGCUGACAGU
CUGGAACAGGGUGUGGAUUCAAGAAAACCCAUGGAUGGAAGACAAAACUCCAGUGGAAACA
UGGGAGGAAAUCCCAUACUUGGGGAAAAGAGAAGACCAAUGGUGCGGCUCAUUGAUUGGGU
UAACAAGCAGGGCCACCUGGGCAAAGAACAUCCAAGCAGCAAUAAAUCAAGUUAGAUCCCU
UAUAGGCAAUGAAGAAUACACAGAUUACAUGCCAUCCAUGAAAAGAUUCAGAAGAGAAGAG
GAAGAAGCAGGAGUUCUGUGGUAGAAAGCAAAACUAACAUGAAACAAGGCUAGAAGUCAGG
UCGGAUUAAGCCAUAGUACGGAAAAAACUAUGCUACCUGUGAGCCCCGUCCAAGGACGUUA
AAAGAAGUCAGGCCAUCAUAAAUGCCAUAGCUUGAGUAAACUAUGCAGCCUGUAGCUCCAC
CUGAGAAGGUGUAAAAAAUCCGGGAGGCCACAAACCAUGGAAGCUGUACGCAUGGCGUAGU
GGACUAGCGGUUAGGGGAGACCCCUCCCUUACAAAUCGCAGCAACAAUGGGGGCCCAAGGC
GAGAUGAAGCUGUAGUCUCGCUGGAAGGACUAGAGGUUAGAGGAGACCCCCCCGAAACAAA
AAACAGCAUAUUGACGCUGGGAAAGACCAGAGAUCCUGCUGUCUCCUCAGCAUCAUUCCAG
GCACAGAACGCCAGAAAAUGGAAUGGUGCUGUUGAAUCAACAGGUUCU

>VDV2 prME nucleotide sequence (SEQ ID NO: 25)

UUCCAUUUAACCACACGUAACGGAGAACCACACAUGAUCGUCAGCAGACAAGAGAAAGGGA
AAAGUCUUCUGUUUAAAACAGAGGUUGGCGUGAACAUGUGUACCCUCAUGGCCAUGGACCU
UGGUGAAUUGUGUGAAGACACAAUCACGUACAAGUGUCCCCUUCUCAGGCAGAAUGAGCCA
GAAGACAUAGACUGUUGGUGCAACUCUACGUCCACGUGGGUAACUUAUGGGACGUGUACCA
CCAUGGGAGAACAUAGAAGAGAAAAAAGAUCAGUGGCACUCGUUCCACAUGUGCGAAUGGG
ACUGGAGACACGAACUGAAACAUGGAUGUCAUCAGAAGGGGCCUGGAAACAUGUCCAGAGA
AUUGAAACUUGGAUCUUGAGACAUCCAGGCUUCACCAUGAUGGCAGCAAUCCUGGCAUACA
CCAUAGGAACGACACAUUUCCAAAGAGCCCUGAUUUUCAUCUUACUGACAGCUGUCACUCC
UUCAAUGACAAUGCGUUGCAUAGGAAUGUCAAAUAGAGACUUUGUGGAAGGGGUUUCAGGA
GGAAGCUGGGUUGACAUAGUCUUAGAACAUGGAAGCUGUGUGACGACGAUGGCAAAAAACA
AACCAACAUUGGAUUUUGAACUGAUAAAAACAGAAGCCAAACAGCCUGCCACCCUAAGGAA
GUACUGUAUAGAGGCAAAGCUAACCAACACAACAACAGAAUCUCGCUGCCCAACACAAGGG
GAACCCAGCCUAAAUGAAGAGCAGGACAAAAGGUUCGUCUGCAAACACUCCAUGGUAGACA
GAGGAUGGGGAAAUGGAUGUGGACUAUUUGGAAAGGGAGGCAUUGUGACCUGUGCUAUGUU
CAGAUGCAAAAAGAACAUGGAAGGAAAAGUUGUGCAACCAGAAAACUUGGAAUACACCAUU
GUGAUAACACCUCACUCAGGGGAAGAGCAUGCAGUCGGAAAUGACACAGGAAAACAUGGCA
AGGAAAUCAAAAUAACACCACAGAGUUCCAUCACAGAAGCAGAAUUGACAGGUUAUGGCAC
UGUCACAAUGGAGUGCUCUCCAAGAACGGGCCUCGACUUCAAUGAGAUGGUGUUGCUGCAG
AUGGAAAAUAAAGCUUGGCUGGUGCACAGGCAAUGGUUCCUAGACCUGCCGUUACCAUGGU
UGCCCGGAGCGGACACACAAGAGUCAAAUUGGAUACAGAAGGAGACAUUGGUCACUUUCAA
AAAUCCCCAUGCGAAGAAACAGGAUGUUGUUGUUUUAGGAUCCCAAGAAGGGGCCAUGCAC
ACAGCACUUACAGGGGCCACAGAAAUCCAAAUGUCAUCAGGAAACUUACUCUUCACAGGAC
AUCUCAAGUGCAGGCUGAGAAUGGACAAGCUACAGCUCAAAGGAAUGUCAUACUCUAUGUG

CACAGGAAAGUUUAAAGUUGUGAAGGAAAUAGCAGAAACACAACAUGGAACAAUAGUUAUC
AGAGUGCAAUAUGAAGGGGACGGCUCUCCAUGCAAGAUCCCUUUUGAGAUAAUGGAUUUGG
AAAAAAGACAUGUCUUAGGUCGCCUGAUUACAGUCAACCCAAUUGUGACAGAAAAAGAUAG
CCCAGUCAACAUAGAAGCAGAACCUCCAUUUGGAGACAGCUACAUCAUCAUAGGAGUAGAG
CCGGGACAACUGAAGCUCAACUGGUUUAAGAAAGGAAGUUCUAUCGGCCAAAUGUUUGAGA
CAACAAUGAGGGGGGCGAAGAGAAUGGCCAUUUUAGGUGACACAGCCUGGGAUUUUGGAUC
CUUGGGAGGAGUGUUUACAUCUAUAGGAAAGGCUCUCCACCAAGUCUUUGGAGCAAUCUAU
GGAGCUGCCUUCAGUGGGGUUUCAUGGACUAUGAAAAUCCUCAUAGGAGUCAUUAUCACAU
GGAUAGGAAUGAAUUCACGCAGCACCUCACUGUCUGUGACACUAGUAUUGGUGGGAAUUGU
GACACUGUAUUUGGGAGUCAUGGUGCAGGCC

>VDV2 E protein sequence (SEQ ID NO: 26)

MRCIGMSNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCI
EAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFRCK
KNMEGKVVQPENLEYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTM
ECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADTQESNWIQKETLVTFKNPH
AKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTGK
FKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGG
VFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVTLVLVGIVTLY
LGVMVQA

>VDV2 M protein sequence (SEQ ID NO: 27)

SVALVPHVRMGLETRTETWMSSEGAWKHVQRIETWILRHPGFTMMAAILAYTIGTTHFQRA
LIFILLTAVTPSMT

Study design

In an open, randomised, controlled, phase IIa trial, 150 healthy adults aged 18-45 years were enrolled at two centres in Mexico City, which is a dengue non-endemic area. Main exclusion criteria were: pregnancy or breast-feeding, human immunodeficiency virus, hepatitis B or C seropositivity, immunodeficiency or any other chronic illness that could interfere with the results, previous residence in or travel of >2 weeks to areas with high dengue endemicity, a history of flavivirus infection or previous vaccination against flavivirus disease. Women who were capable of conceiving were required to use an effective method of contraception or abstinence for at least 4 weeks before the first injection until at least four weeks after the last injection.

Participants were randomised into two groups and vaccinations were performed on Day 0 and Day 105 (±15 days). The groups received the following formulations:

Group 1: Blended CYD/VDV2 tetravalent formulation, i.e. a formulation comprising CYD-1, CYD-3, CYD4 and VDV2.

Group 2: Control tetravalent formulation (CYD-TDV), i.e. CYD-1, CYD-2, CYD-3 and CYD-4.

The formulations contained 10^5 $CCID_{50}$ of each serotype of the CYD viruses and the formulation administered to Group 1 contained 10^4 $CCID_{50}$ of the VDV-2 virus.

Viremia

To evaluate the safety of the vaccines, the presence of CYD-1–4 or VDV-2 was assessed in serum collected 7, 14 and 21 days after each injection. Analyses were performed by the Global Clinical Immunology laboratory (Sanofi Pasteur, Swiftwater, PA, USA).

Analyses for CYD-1–4 viremia were performed in two steps, as previously described in Poo *et al.*, Pediatr Infect Dis J (2011) 30: e9. Briefly, a first, non-serotype-specific, reverse transcriptase-polymerase chain reaction (RT-PCR) was used to detect the presence of any of the four CYD viruses. Samples that were positive in this first test were then analysed using four CYD serotype-specific quantitative RT-PCRs. In the non-serotype-specific RT-PCR, RNA was extracted from the serum using a commercial kit and an RT-PCR was carried out with primers from the yellow fever core gene sequence. In the serotype-specific RT-PCRs, RNA was again extracted from the serum using a commercial kit and an RT-PCR was carried out with serotype-specific primers from the envelope non-structural protein 1 junction gene sequence for each serotype. A dengue RT-PCR for serotype 2 was performed in group 1 since the tetravalent blending formulation administered to this group contained the VDV-2 virus.

Immunogenicity

Antibody levels to each of the four dengue virus serotypes were determined by 50% plaque reduction neutralisation test on serum collected 28 days after each injection as well as on day 365 after the first injection. Briefly, serial 2-fold dilutions of heat-inactivated serum were mixed with a constant challenge dose of each dengue serotype DEN-1, -2, -3, or -4 (expressed as plaque forming unit [PFU]/mL). The mixtures were inoculated into wells of a 24-well plate of confluent VERO cell monolayers. After incubation for several days, dengue virus infection is indicated by formation of plaques. The neutralising antibody titre is calculated as the highest reciprocal dilution (1/dil) of serum at which ≥50% reduction in viral plaque count is observed (PRNT50). The lower limit of quantitation of the dengue PRNT50 is 10; samples with titres ≥10 were considered seropositive.

Results

Formulations were administered to participants in Groups 1 and 2 on day 0 and day 105 of the study. There were no marked differences between the two groups with regard to the injection site or systemic reactogenicity after either the first or the second vaccination. Viremia was assessed in serum collected 7, 14 and 21 days after each injection (Table 7). The neutralising antibody titres were measured 28 days after each injection and on day 365 after the first injection (Table 8).

Table 7. Vaccine virus viremia 7, 14, or 21 days after first and second injections (n (%) with detectable and quantifiable viremia)

	First injection		Second injection	
	Group 1 Blended CYD/VDV	Group 2 Tetravalent CYD-TDV	Group 1 Blended CYD/VDV	Group 2 Tetravalent CYD-TDV
Non-serotype specific				
N	29	31	28	29
Detectable viraemia	27 (93%)	25 (81%)	1 (4%)	1 (3%)
Quantifiable viraemia	1 (3%)	2 (6%)	0	0
DENV-1				
Detectable viraemia	1 (3%)	4 (13%)	0	0
Quantifiable viraemia	0	2 (7%)	0	0
DENV-2				
Detectable viraemia	0	2 (6%)	0	0
Quantifiable viraemia	0	0	0	0
DENV-3				
Detectable viraemia	8 (28%)	7 (23%)	1 (4%)	0
Quantifiable viraemia	0	0	0	0
DENV-4				
Detectable viraemia	24 (83%)	21 (68%)	0	0
Quantifiable viraemia	0	3 (1%)	0	0

After the first injection, detectable viremia, as determined by the non-serotype specific RT-PCR test, was observed in a similar proportion of participants in both groups (see Table 7). In the majority of cases, viremia was below the lower limit of quantitation. Analysis with the serotype-specific assays showed that CYD-4 was the most commonly detected serotype, followed by CYD-3. After the second injection of the blended CYD/VDV

vaccine in Group 1 or the CYD-TDV vaccine in Group 2, viremia was only detected in one participant per group by the non-serotype-specific assay.

Accordingly, there was no significant difference between the levels of viremia induced by the blended CYD/VDV and CYD-TDV.

Table 8. Geometric mean titres (95% confidence interval) of dengue antibodies 28 days after the first and second injections and 365 days after the first injection

	Group 1 CYD/VDV blended	Group 2 CYD-TDV
First injection		
Serotype 1	15 (9;28)	17 (10;31)
Serotype 2	17 (8;33)	32 (16;65)
Serotype 3	64 (31;133)	23 (13;39)
Serotype 4	552 (299;1019)	468 (226;968)
Second injection		
Serotype 1	54 (30;96)	28 (15;50)
Serotype 2	152 (79;293)	43 (23;79)
Serotype 3	127 (71;229)	46 (29;73)
Serotype 4	246 (159;382)	173 (97;307)
365 days post-dose 1		
Serotype 1	14 (9;22)	18 (10;30)
Serotype 2	55 (32;94)	16 (9;29)
Serotype 3	36 (20;64)	11 (7;16)
Serotype 4	103 (69;155)	72 (44;117)

It can be seen from Table 8 that the second injection of the blended CYD/VDV vaccine (Group 1) induced higher GMTs against serotype 2 of dengue virus than the CYD-TDV vaccine (Group 2). An improved response to serotype 2 in the blended CYD/VDV group was also observed 365 days after the first dose.

Furthermore, the second injection of the blended CYD/VDV vaccine (Group 1) resulted in an improved neutralising antibody response against all serotypes of dengue virus when compared with the group receiving the CYD-TDV vaccine (Group 2). Importantly, the blended CYD/VDV formulation group demonstrated a more persistent neutralising antibody response against dengue virus than the CYD-TDV group on day 365 after the first injection.

The example therefore shows that, overall, the blended CYD-1, 3, 4/VDV2 vaccine formulation induces stronger and longer lasting immune responses against the dengue virus serotypes than the CYD-TDV vaccine while showing a similar safety profile, as determined by the levels of viremia.

Sequence Listing

SEQ ID NO.	Sequence
1	prM+E CYD23 circulating strain nucleotide sequence
2	prM+E CYD23 circulating strain protein sequence
3	prM+E consensus serotype 2 protein sequence
4	prM+E LAV2 nucleotide sequence
5	prM+E BID/V585 nucleotide sequence
6	prM+E PR/DB023 nucleotide sequence
7	prM+E MD1280 nucleotide sequence
8	prM+E LAV2 protein sequence
9	prM+E BID/V585 protein sequence
10	prM+E PR/DB023 protein sequence
11	prM+E MD1280 protein sequence
12	E consensus serotype 2 protein sequence
13	E LAV2 protein sequence
14	E BID/V585 protein sequence
15	E PR/DB023 protein sequence
16	E MD1280 protein sequence
17	M consensus serotype 2 protein sequence
18	E CYD23 circulating strain protein sequence
19	M LAV2 protein sequence
20	M BID/V585 protein sequence
21	M PR/DB023 protein sequence
22	M MD1280 protein sequence
23	M CYD23 circulating strain protein sequence
24	Entire nucleotide sequence of VDV2 (RNA equivalent)
25	prM+E VDV2 nucleotide sequence (RNA equivalent)
26	E VDV2 protein sequence
27	M VDV2 protein sequence

In the listed nucleotide sequences, where a nucleotide sequence is DNA, the nucleotide T may be replaced with the nucleotide U to give the RNA equivalent of that DNA sequence. Similarly, where a nucleotide sequence is RNA, the nucleotide U may be replaced by the nucleotide T to give the equivalent DNA sequence. The DNA sequences listed above constitute the cDNA sequences of the noted dengue viruses and therefore the equivalent RNA sequences constitute the positive strand RNA of those dengue viruses.

CLAIMS

1. A dengue virus serotype 2 vaccine composition comprising:

(i) a dengue antigen selected from the group consisting of:
(a) a live attenuated dengue virus;
(b) an inactivated dengue virus;
(c) a live attenuated or inactivated chimeric dengue virus;
(d) a dengue virus-like particle (VLP); and
(e) a combination of two or more of (a) to (d);

or

(iii) a nucleic acid construct or viral vector which is able to express in a human cell a dengue antigen which is a dengue VLP;

wherein said dengue antigen comprises a polypeptide having at least 90% identity to SEQ ID NO: 12.

2. A composition as claimed in claim 1, wherein said polypeptide comprises a valine residue at the position within the polypeptide that corresponds to position 251 of SEQ ID NO: 12.

3. A composition as claimed in claim 1 or claim 2, wherein said polypeptide comprises a methionine residue at the position within the polypeptide that corresponds to position 6 of SEQ ID NO: 12.

4. A composition as claimed in any preceding claim, wherein said polypeptide comprises a valine residue at the position within the polypeptide that corresponds to position 129 of SEQ ID NO: 12.

5. A composition as claimed in any preceding claim, wherein said polypeptide comprises an isoleucine residue at the position within the polypeptide that corresponds to position 141 of SEQ ID NO: 12.

6. A composition as claimed in any preceding claim, wherein said polypeptide comprises an isoleucine residue at the position within the polypeptide that corresponds to position 164 of SEQ ID NO: 12.

7. A composition as claimed in any preceding claim, wherein said polypeptide comprises an aspartate residue at the position within the polypeptide that corresponds to position 203 of SEQ ID NO: 12.

8. A composition as claimed in any preceding claim, wherein said polypeptide comprises a threonine residue at the position within the polypeptide that corresponds to position 226 of SEQ ID NO: 12.

9. A composition as claimed in any preceding claim, wherein said polypeptide comprises a glycine residue at the position within the polypeptide that corresponds to position 228 of SEQ ID NO: 12.

10. A composition as claimed in any preceding claim, wherein said polypeptide comprises an isoleucine residue at the position within the polypeptide that corresponds to position 308 of SEQ ID NO: 12.

11. A composition as claimed in any preceding claim, wherein said polypeptide comprises a threonine residue at the position within the polypeptide that corresponds to position 478 of SEQ ID NO: 12.

12. A composition as claimed in any preceding claim, wherein said polypeptide comprises an isoleucine residue at the position within the polypeptide that corresponds to position 484 of SEQ ID NO: 12.

13. A composition as claimed in any preceding claim, wherein said polypeptide comprises an isoleucine residue at the position within the polypeptide that corresponds to position 485 of SEQ ID NO: 12.

14. A composition as claimed in any preceding claim, wherein said polypeptide comprises an alanine residue at the position within the polypeptide that corresponds to position 491 of SEQ ID NO: 12.

15. A composition as claimed in any preceding claim, wherein said dengue antigen comprises a polypeptide having at least 90% identity to SEQ ID NO: 3.

16. A composition as claimed in any one of claims 1 to 14, wherein said polypeptide has at least 90% identity to SEQ ID NO: 3.

17. A composition as claimed in claim 15 or 16, wherein said polypeptide comprises a glycine residue at the position within the polypeptide that corresponds to position 15 of SEQ ID NO: 3.

18. A composition as claimed in any one of claims 15 to 17, wherein said polypeptide comprises a leucine residue at the position within the polypeptide that corresponds to position 24 of SEQ ID NO: 3.

19. A composition as claimed in any one of claims 15 to 18, wherein said polypeptide comprises an isoleucine residue at the position within the polypeptide that corresponds to position 39 of SEQ ID NO: 3.

20. A composition as claimed in any one of claims 15 to 19, wherein said polypeptide comprises a valine residue at the position within the polypeptide that corresponds to position 120 of SEQ ID NO: 3.

21. A composition as claimed in any one of claims 15 to 20, wherein said polypeptide comprises a threonine residue at the position within the polypeptide that corresponds to position 125 of SEQ ID NO: 3.

22. A composition as claimed in any one of claims 1 to 21, wherein said polypeptide comprises: (i) the sequence as set forth in SEQ ID NO: 13 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 13; (ii) the sequence as set forth in SEQ ID NO: 14 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 14; (iii) the sequence as set forth in SEQ ID NO: 15 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 15; (iv) the sequence as set forth in SEQ ID NO: 16 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 16; (v) the sequence as set forth in SEQ ID NO: 18 or a sequence

having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 18 or (vi) the sequence as set forth in SEQ ID NO: 26 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 26.

23. A composition as claimed in claim 1, wherein said dengue antigen comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 13; SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 26.

24. A composition as claimed in claim 23, wherein said dengue antigen comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 13 and SEQ ID NO: 16.

25. A composition as claimed in claim 22, wherein said dengue antigen further comprises a polypeptide comprising: (i) the sequence as set forth in SEQ ID NO: 19 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 19; (ii) the sequence as set forth in SEQ ID NO: 20 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 20; (iii) the sequence as set forth in SEQ ID NO: 21 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 21; (iv) the sequence as set forth in SEQ ID NO: 22 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 22; (v) the sequence as set forth in SEQ ID NO: 23 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 23; (vi) the sequence as set forth in SEQ ID NO: 27 or a sequence having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 27.

26. A composition as claimed in claim 23, wherein said dengue antigen further comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 19; SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 27.

27. A composition as claimed in claim 22, wherein said dengue antigen comprises:

i) a polypeptide having the sequence as set forth in SEQ ID NO: 13 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 13; and
a polypeptide having the sequence as set forth in SEQ ID NO: 19 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 19;

ii) a polypeptide having the sequence as set forth in SEQ ID NO: 14 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 14; and
a polypeptide having the sequence as set forth in SEQ ID NO: 20 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 20;

iii) a polypeptide having the sequence as set forth in SEQ ID NO: 15 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 15; and
a polypeptide having the sequence as set forth in SEQ ID NO: 21 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 21;

iv) a polypeptide having the sequence as set forth in SEQ ID NO: 16 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 16; and
a polypeptide having the sequence as set forth in SEQ ID NO: 22 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 22;

v) a polypeptide having the sequence as set forth in SEQ ID NO: 18 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 18; and
a polypeptide having the sequence as set forth in SEQ ID NO: 23 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 23; or

vi) a polypeptide having the sequence as set forth in SEQ ID NO: 26 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 26; and

a polypeptide having the sequence as set forth in SEQ ID NO: 27 or a polypeptide having at least 1 and no more than 5 amino acid substitutions with respect to the sequence as set forth in SEQ ID NO: 27.

28. A composition as claimed in claim 26, wherein said dengue antigen comprises: i) a polypeptide of SEQ ID NO: 13 and a polypeptide of SEQ ID NO: 19; ii) a polypeptide of SEQ ID NO: 14 and a polypeptide of SEQ ID NO: 20; iii) a polypeptide of SEQ ID NO: 15 and a polypeptide of SEQ ID NO: 21; iv) a polypeptide of SEQ ID NO: 16 and a polypeptide of SEQ ID NO: 22; v) a polypeptide of SEQ ID NO: 18 and a polypeptide of SEQ ID NO: 23 or vi) a polypeptide of SEQ ID NO: 26 and a polypeptide of SEQ ID NO: 27.

29. A composition as claimed in claim 15 or claim 16, wherein said dengue antigen comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 8; SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11.

30. A composition as claimed in claim 29, wherein said dengue antigen comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 8; and SEQ ID NO: 11.

31. A composition as claimed in any preceding claim, wherein said dengue antigen is selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus; or (d) a combination of two or more of (a) to (c); wherein said dengue antigen comprises a nucleotide sequence encoding a protein comprising a polypeptide as defined in any one of claims 1 to 30.

32. A vaccine composition comprising a dengue antigen of serotype 2 selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus; or (d) a combination of two or more of (a) to (c); wherein said dengue antigen comprises a nucleotide sequence encoding a protein comprising the polypeptide or polypeptides as defined in any one of claims 1 to 30.

33. A vaccine composition comprising a dengue antigen of serotype 2 selected from the group consisting of: (a) a live attenuated dengue virus; (b) an inactivated dengue virus; (c) a live attenuated or inactivated chimeric dengue virus; or (d) a

combination of two or more of (a) to (c); wherein said dengue antigen comprises a nucleotide sequence having at least 90% sequence identity to a sequence selected from the group consisting of the RNA equivalent of SEQ ID NO: 1, the RNA equivalent of SEQ ID NO: 4, the RNA equivalent of SEQ ID NO: 5, the RNA equivalent of SEQ ID NO: 6, the RNA equivalent of SEQ ID NO: 7 and SEQ ID NO: 25.

34. A composition as claimed in any preceding claim, wherein said composition comprises a live attenuated chimeric dengue virus.

35. A composition as claimed in claim 34, wherein said composition comprises one or more proteins from a dengue virus and one or more proteins from a different flavivirus.

36. A composition as claimed in claim 35, wherein the different flavivirus is a yellow fever virus.

37. A composition as claimed in claim 36, wherein the yellow fever virus is YF-Vax.

38. A composition as claimed in any preceding claim, wherein the composition further comprises a dengue antigen of serotype 1, a dengue antigen of serotype 3 and a dengue antigen of serotype 4.

39. A composition as claimed in claim 38, wherein said dengue antigens of serotypes 1, 3 and 4 are each independently selected from the group consisting of a live attenuated dengue virus and a live attenuated chimeric dengue virus.

40. A composition as claimed in claim 39, wherein said dengue antigens of serotypes 1, 3 and 4 are each a live attenuated chimeric dengue virus in which the genetic backbone of a recipient flavivirus has been modified by exchanging the sequences encoding the prM and E proteins of the recipient flavivirus with the corresponding sequences of a dengue virus.

41. A composition as claimed in claim 40, wherein the recipient flavivirus is a yellow fever virus.

42. A composition as claimed in any one of claims 38 to 41, wherein said dengue antigens of serotypes 1, 3 and 4 are each a live attenuated chimeric dengue virus and the dengue antigen of serotype 2 is a live attenuated dengue virus of serotype 2 which comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO: 24.

43. A pharmaceutical formulation comprising a composition as claimed in any preceding claim and a pharmaceutically acceptable carrier, diluent or excipient.

44. A composition as claimed in any one of claims 1 to 42 for use in therapy.

45. A composition as claimed in any one of claims 1 to 42 for use in a method of protecting a human subject against dengue disease caused by a dengue virus of serotype 2.

46. A composition as claimed in any one of claims 38 to 42 for use in a method of protecting a human subject against dengue disease caused by a dengue virus of serotype 1, serotype 2, serotype 3 or serotype 4.

47. A method of protecting a human subject against dengue disease caused by a dengue virus of serotype 2, wherein said method comprises administering to said subject an effective amount of a composition according to any one of claims 1 to 42.

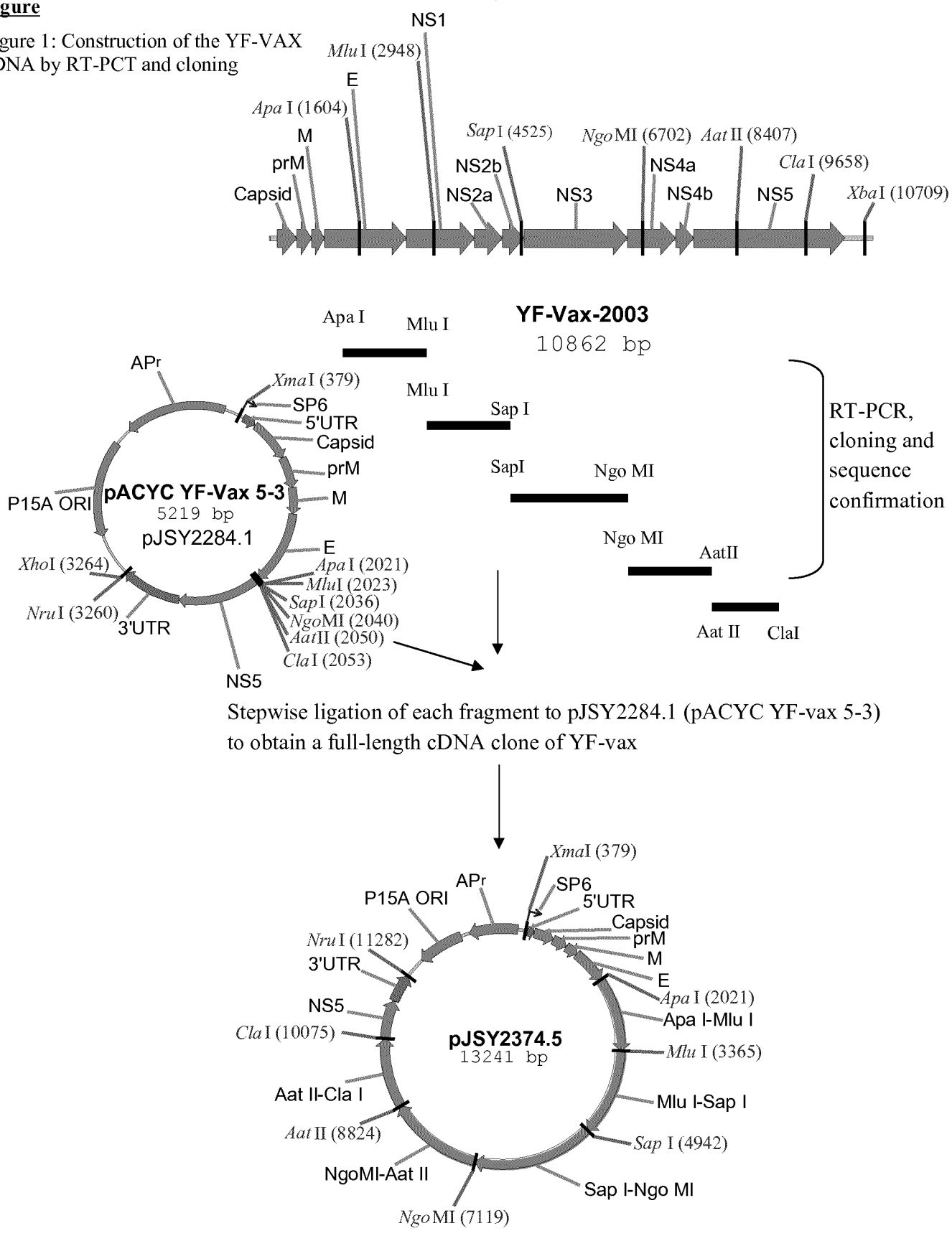
48. A method of protecting a human subject against dengue disease caused by a dengue virus of serotype 1, serotype 2, serotype 3 or serotype 4, wherein said method comprises administering to said subject an effective amount of a composition according to any one of claims 38 to 42.

49. A kit comprising a composition according to any one of claims 1 to 42 and instructions for use of said composition in a method of protecting a human subject against dengue disease caused by a dengue virus of serotype 2.

50. A kit comprising a composition according to any one of claims 38 to 42 and instructions for use of said composition in a method of protecting a human subject against dengue disease caused by a dengue virus of serotype 1, serotype 2, serotype 3 or serotype 4.

Figure

Figure 1: Construction of the YF-VAX cDNA by RT-PCT and cloning



INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/065669

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-14(completely); 22-28, 31-50(partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/065669

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 A61K39/295
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JORGE E OSORIO ET AL: "Development of DENVax: A chimeric dengue-2 PDK-53-based tetravalent vaccine for protection against dengue fever", VACCINE, vol. 29, no. 42, 2011, - 21 July 2011 (2011-07-21), pages 7251-7260, XP028285284, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2011.07.020 [retrieved on 2011-07-11] the whole document -----	1-14, 31-50
X	WO 2011/146933 A2 (UNIV PITTSBURGH [US]; ROSS TED M [US]; VASILAKIS NIKOLAOS [US]) 24 November 2011 (2011-11-24) the whole document -----	1-14, 22-28, 31-50

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 December 2013	18/12/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Hermann, Patrice

4

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BHAMARAPRAVATI N ET AL: "Live attenuated tetravalent dengue vaccine", VACCINE, ELSEVIER LTD, GB, vol. 18, 1 January 2000 (2000-01-01), pages 44-47, XP002160013, ISSN: 0264-410X, DOI: 10.1016/S0264-410X(00)00040-2	1-14, 31-33, 38-40, 42-50
A	the whole document	34-37,41
X	Bruno Guy: "Development of Sanofi Pasteur tetravalent dengue vaccine", , vol. 6, no. 9 1 September 2010 (2010-09-01), pages 696-705, XP055046419, DOI: DOI: 10.4161.hv.6.9.12739 Retrieved from the Internet: URL:http://www.orphanresearch.com/journals/vaccines/GuyHV6-9.pdf [retrieved on 2012-12-03] the whole document	1-14, 31-50
X	SHUO ZHANG ET AL: "Vaccination with dengue virus-like particles induces humoral and cellular immune responses in mice", VIROLOGY JOURNAL, BIOMED CENTRAL, LONDON, GB, vol. 8, no. 1, 30 June 2011 (2011-06-30), page 333, XP021103960, ISSN: 1743-422X, DOI: 10.1186/1743-422X-8-333	1-14, 31-33, 38-40, 42-50
A	the whole document	34-37,41
A	CLEMENTS D E ET AL: "Development of a recombinant tetravalent dengue virus vaccine: Immunogenicity and efficacy studies in mice and monkeys", VACCINE, ELSEVIER LTD, GB, vol. 28, no. 15, 24 March 2010 (2010-03-24), pages 2705-2715, XP026946252, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2010.01.022 [retrieved on 2010-03-08] the whole document	1-14, 31-50

-/--

4

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TWIDDY S S ET AL: "Phylogenetic Relationships and Differential Selection Pressures among Genotypes of Dengue-2 Virus", VIROLOGY, ACADEMIC PRESS,ORLANDO, US, vol. 298, no. 1, 20 June 2002 (2002-06-20) , pages 63-72, XP004469470, ISSN: 0042-6822, DOI: 10.1006/VIRO.2002.1447 the whole document	1-14, 31-50
X	WO 2011/013097 A2 (REHM BERND HELMUT ADAM [NZ]; PARLANE NATALIE ANNE [NZ]; WEDLOCK DAVID) 3 February 2011 (2011-02-03) the whole document	22-28, 31-50
X	WO 2012/051491 A1 (US OF AMERICA AS REPRESENTED BY THE SECRETARY NAT INST OF HEALTH [US];) 19 April 2012 (2012-04-19) the whole document	22-28, 31-50
X	WO 02/081754 A1 (US GOV HEALTH & HUMAN SERV [US]; CHANG GWONG-JEN J [US]) 17 October 2002 (2002-10-17) the whole document	22-28, 31-50
A	EP 1 958 959 A2 (CT INGENIERIA GENETICA BIOTECH [CU]) 20 August 2008 (2008-08-20) the whole document	22-28, 31-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/065669

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011146933 A2	24-11-2011	CN 102906266 A	30-01-2013
		EP 2571990 A2	27-03-2013
		US 2013071419 A1	21-03-2013
		WO 2011146933 A2	24-11-2011
WO 2011013097 A2	03-02-2011	AU 2010277222 A1	08-03-2012
		CA 2769645 A1	03-02-2011
		CN 102573891 A	11-07-2012
		EA 201290072 A1	28-12-2012
		EP 2461822 A2	13-06-2012
		JP 2013500329 A	07-01-2013
		SG 178144 A1	29-03-2012
		US 2012201846 A1	09-08-2012
		WO 2011013097 A2	03-02-2011
WO 2012051491 A1	19-04-2012	NONE	
WO 02081754 A1	17-10-2002	BR 0208301 A	09-03-2004
		CA 2443323 A1	17-10-2002
		CN 1500152 A	26-05-2004
		CN 101002936 A	25-07-2007
		EP 1383931 A1	28-01-2004
		EP 1935991 A2	25-06-2008
		EP 2332572 A1	15-06-2011
		HK 1062836 A1	05-10-2007
		JP 4448281 B2	07-04-2010
		JP 5236597 B2	17-07-2013
		JP 2004532023 A	21-10-2004
		JP 2010017185 A	28-01-2010
		MX PA03008838 A	15-10-2004
		NZ 529106 A	24-03-2005
		US 2003022849 A1	30-01-2003
		US 2005163804 A1	28-07-2005
		US 2007166329 A1	19-07-2007
		US 2007166701 A1	19-07-2007
		US 2010003273 A1	07-01-2010
		US 2010040643 A1	18-02-2010
		WO 02081754 A1	17-10-2002
		ZA 200307580 A	03-01-2005
EP 1958959 A2	20-08-2008	AR 058215 A1	23-01-2008
		AU 2006317350 A1	31-05-2007
		BR PI0618865 A2	13-09-2011
		CA 2630629 A1	31-05-2007
		CN 101360758 A	04-02-2009
		CU 23586 A1	30-10-2010
		EP 1958959 A2	20-08-2008
		JP 2009524581 A	02-07-2009
		KR 20080080137 A	02-09-2008
		US 2009312190 A1	17-12-2009
		WO 2007059715 A2	31-05-2007
		ZA 200804874 A	29-04-2009

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14(completely); 31-50(partially)

   Invention 1 relates to a dengue virus serotype 2 vaccine composition comprising a dengue antigen in the form of (a) a live attenuated dengue virus, (b) an inactivated dengue virus, (c) a live attenuated or inactivated chimeric dengue virus, or (d) a dengue virus like particle or a nucleic acid construct or viral vector encoding the same in human cell, wherein the dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 12; and uses thereof in the prevention of human subjects against dengue disease caused by a dengue virus serotype 2, and kit comprising said vaccine composition.

   ---

2. claims: 15-21, 29, 30(completely); 31-50(partially)

   Invention 2 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 3.

   ---

3. claims: 22-28, 31-50(all partially)

   Invention 3 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 13.

   ---

4. claims: 22, 23, 25-28, 31-50(all partially)

   Invention 4 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 14.

   ---

5. claims: 22, 23, 25-28, 31-50(all partially)

   Invention 5 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 15.

   ---

6. claims: 22-28, 31-50(all partially)

   Invention 6 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 16.

   ---

7. claims: 22, 23, 25-28, 31-50(all partially)

Invention 7 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 18.

---

8. claims: 22, 23, 25-28, 31-50(all partially)

Invention 8 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 18.

---

9. claims: 29-50(partially)

Invention 9 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 8.

---

10. claims: 29, 31-50(all partially)

Invention 10 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 9.

---

11. claims: 29, 31-50(all partially)

Invention 11 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 10.

---

12. claims: 29-50(partially)

Invention 12 is identical to invention 1 when said dengue antigen comprises a polypeptide having at least 90% identity to SEQ. ID. NO: 11.

---

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 104812408 A
(43)申请公布日 2015.07.29

(21)申请号 201380049741.2

(22)申请日 2013.07.24

(30)优先权数据
12305908.1 2012.07.24 EP
12305911.5 2012.07.25 EP

(85)PCT国际申请进入国家阶段日
2015.03.24

(86)PCT国际申请的申请数据
PCT/EP2013/065669 2013.07.24

(87)PCT国际申请的公布数据
WO2014/016362 EN 2014.01.30

(71)申请人 赛诺菲巴斯德有限公司
地址 法国里昂

(72)发明人 J. 姚 Y. 吉雷尔 - 尚巴兹
I. 勒加斯特卢瓦 N. 曼特尔
V. 巴尔邦 J. 兰 B. 居伊

(74)专利代理机构 中国专利代理(香港)有限公司 72001
代理人 初明明 梁谋

(51)Int.Cl.
A61K 39/12(2006.01)
A61K 39/295(2006.01)

权利要求书5页 说明书62页
序列表71页 附图1页

(54)发明名称

用于防止登革热病毒感染的疫苗组合物

(57)摘要

本发明涉及可用于保护人类受试者免于登革热病的方法的疫苗组合物。

CN 104812408 A

1. 一种登革热病毒血清型 2 疫苗组合物,所述组合物包含 :

(i) 选自以下的登革热抗原 :

(a) 活的减毒登革热病毒 ;

(b) 灭活的登革热病毒 ;

(c) 活的减毒或灭活的嵌合登革热病毒 ;

(d) 登革热病毒样颗粒 (VLP) ;和

(e) (a)-(d) 的两种或更多种的组合 ;

或

(iii) 能够在人细胞中表达登革热抗原的核酸构建体或病毒载体,所述登革热抗原是登革热 VLP ;

其中所述登革热抗原包含与 SEQ ID NO: 12 具有至少 90% 同一性的多肽。

2. 权利要求 1 的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 251 位的多肽内的位置上包含缬氨酸残基。

3. 权利要求 1 或权利要求 2 的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 6 位的多肽内的位置上包含甲硫氨酸残基。

4. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 129 位的多肽内的位置上包含缬氨酸残基。

5. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 141 位的多肽内的位置上包含异亮氨酸残基。

6. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 164 位的多肽内的位置上包含异亮氨酸残基。

7. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 203 位的多肽内的位置上包含天冬氨酸残基。

8. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 226 位的多肽内的位置上包含苏氨酸残基。

9. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 228 位的多肽内的位置上包含甘氨酸残基。

10. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 308 位的多肽内的位置上包含异亮氨酸残基。

11. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 478 位的多肽内的位置上包含苏氨酸残基。

12. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 484 位的多肽内的位置上包含异亮氨酸残基。

13. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 485 位的多肽内的位置上包含异亮氨酸残基。

14. 前述权利要求中任一项的组合物,其中所述多肽在相当于 SEQ ID NO: 12 的 491 位的多肽位的内置上包含丙氨酸残基。

15. 前述权利要求中任一项的组合物,其中所述登革热抗原包含与SEQ ID NO: 3具有至少 90% 同一性的多肽。

16. 权利要求 1-14 中任一项的组合物，其中所述多肽与 SEQ ID NO: 3 具有至少 90% 同一性。

17. 权利要求 15 或 16 的组合物，其中所述多肽在相当于 SEQ ID NO: 3 的 15 位的多肽内的位置上包含甘氨酸残基。

18. 权利要求 15-17 中任一项的组合物，其中所述多肽在相当于 SEQ ID NO: 3 的 24 位的多肽内的位置上包含亮氨酸残基。

19. 权利要求 15-18 中任一项的组合物，其中所述多肽在相当于 SEQ ID NO: 3 的 39 位的多肽内的位置上包含异亮氨酸残基。

20. 权利要求 15-19 中任一项的组合物，其中所述多肽在相当于 SEQ ID NO: 3 的 120 位的多肽内的位置上包含缬氨酸残基。

21. 权利要求 15-20 中任一项的组合物，其中所述多肽在相当于 SEQ ID NO: 3 的 125 位的多肽内的位置上包含苏氨酸残基。

22. 权利要求 1-21 中任一项的组合物，其中所述多肽包含 :(i) SEQ ID NO: 13 所示序列或相对于 SEQ ID NO: 13 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(ii) SEQ ID NO: 14 所示序列或相对于 SEQ ID NO: 14 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(iii) SEQ ID NO: 15 所示序列或相对于 SEQ ID NO: 15 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(iv) SEQ ID NO: 16 所示序列或相对于 SEQ ID NO: 16 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(v) SEQ ID NO: 18 所示序列或相对于 SEQ ID NO: 18 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列，或 (vi) SEQ ID NO: 26 所示序列或相对于 SEQ ID NO: 26 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列。

23. 权利要求 1 的组合物，其中所述登革热抗原包含含有选自以下序列的多肽 :SEQ ID NO: 13、SEQ ID NO: 14、SEQ ID NO: 15、SEQ ID NO: 16、SEQ ID NO: 18 和 SEQ ID NO: 26。

24. 权利要求 23 的组合物，其中所述登革热抗原包含含有选自以下序列的多肽 :SEQ ID NO: 13 和 SEQ ID NO: 16。

25. 权利要求 22 的组合物，其中所述登革热抗原还包含含有以下的多肽 :(i) SEQ ID NO: 19 所示序列或相对于 SEQ ID NO: 19 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(ii) SEQ ID NO: 20 所示序列或相对于 SEQ ID NO: 20 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(iii) SEQ ID NO: 21 所示序列或相对于 SEQ ID NO: 21 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(iv) SEQ ID NO: 22 所示序列或相对于 SEQ ID NO: 22 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(v) SEQ ID NO: 23 所示序列或相对于 SEQ ID NO: 23 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(vi) SEQ ID NO: 27 所示序列或相对于 SEQ ID NO: 27 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列。

26. 权利要求 23 的组合物，其中所述登革热抗原还包含含有选自以下序列的多肽 : SEQ ID NO: 19、SEQ ID NO: 20、SEQ ID NO: 21、SEQ ID NO: 22、SEQ ID NO: 23 和 SEQ ID NO: 27。

27. 权利要求 22 的组合物，其中所述登革热抗原包含 :

i) 具有 SEQ ID NO: 13 所示序列的多肽或相对于 SEQ ID NO: 13 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 19 所示序列的多肽或相对于 SEQ ID NO: 19 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;

ii) 具有 SEQ ID NO: 14 所示序列的多肽或相对于 SEQ ID NO: 14 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 20 所示序列的多肽或相对于 SEQ ID NO: 20 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;

iii) 具有 SEQ ID NO: 15 所示序列的多肽或相对于 SEQ ID NO: 15 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 21 所示序列的多肽或相对于 SEQ ID NO: 21 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;

iv) 具有 SEQ ID NO: 16 所示序列的多肽或相对于 SEQ ID NO: 16 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 22 所示序列的多肽或相对于 SEQ ID NO: 22 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;

v) 具有 SEQ ID NO: 18 所示序列的多肽或相对于 SEQ ID NO: 18 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 23 所示序列的多肽或相对于 SEQ ID NO: 23 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;或

vi) 具有 SEQ ID NO: 26 所示序列的多肽或相对于 SEQ ID NO: 26 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 27 所示序列的多肽或相对于 SEQ ID NO: 27 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽。

28. 权利要求 26 的组合物,其中所述登革热抗原包含 :i) SEQ ID NO: 13 的多肽和 SEQ ID NO: 19 的多肽 ;ii) SEQ ID NO: 14 的多肽和 SEQ ID NO: 20 的多肽 ;iii) SEQ ID NO: 15 的多肽和 SEQ ID NO: 21 的多肽 ;iv) SEQ ID NO: 16 的多肽和 SEQ ID NO: 22 的多肽 ;v) SEQ ID NO: 18 的多肽和 SEQ ID NO: 23 的多肽或 vi) SEQ ID NO: 26 的多肽和 SEQ ID NO: 27 的多肽。

29. 权利要求 15 或权利要求 16 的组合物,其中所述登革热抗原包含含有选自以下序列的多肽 :SEQ ID NO: 8、SEQ ID NO: 9、SEQ ID NO: 10 和 SEQ ID NO: 11。

30. 权利要求 29 的组合物,其中所述登革热抗原包含含有选自以下序列的多肽 :SEQ ID NO: 8 和 SEQ ID NO: 11。

31. 前述权利要求中任一项的组合物,其中所述登革热抗原选自 :(a) 活的减毒登革热病毒 ;(b) 灭活的登革热病毒 ;(c) 活的减毒或灭活的嵌合登革热病毒 ;或 (d) (a)-(c) 的两种或更多种的组合 ;其中所述登革热抗原包含编码包含权利要求 1-30 中任一项限定的多肽的蛋白质的核苷酸序列。

32. 一种疫苗组合物,其包含选自以下血清型 2 的登革热抗原 :(a) 活的减毒登革热病毒 ;(b) 灭活的登革热病毒 ;(c) 活的减毒或灭活的嵌合登革热病毒 ;或 (d) (a)-(c) 的两

种或更多种的组合；其中所述登革热抗原包含编码包含权利要求 1-30 中任一项限定的一种或多种多肽的蛋白质的核苷酸序列。

33. 一种疫苗组合物，其包含选自以下血清型 2 的登革热抗原：(a) 活的减毒登革热病毒；(b) 灭活的登革热病毒；(c) 活的减毒或灭活的嵌合登革热病毒；或 (d) (a)-(c) 的两种或更多种的组合；其中所述登革热抗原包含与选自以下的序列具有至少 90% 序列同一性的核苷酸序列：SEQ ID NO: 1 的 RNA 等同物、SEQ ID NO: 4 的 RNA 等同物、SEQ ID NO: 5 的 RNA 等同物、SEQ ID NO: 6 的 RNA 等同物、SEQ ID NO: 7 的 RNA 等同物和 SEQ ID NO: 25。

34. 前述权利要求中任一项的组合物，其中所述组合物包含活的减毒嵌合登革热病毒。

35. 权利要求 34 的组合物，其中所述组合物包含来自登革热病毒的一种或多种蛋白质和来自不同黄病毒的一种或多种蛋白质。

36. 权利要求 35 的组合物，其中所述不同的黄病毒是黄热病病毒。

37. 权利要求 36 的组合物，其中所述黄热病病毒是 YF-Vax。

38. 前述权利要求中任一项的组合物，其中所述组合物还包含血清型 1 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原。

39. 权利要求 38 的组合物，其中所述血清型 1、3 和 4 的登革热抗原各自独立选自活的减毒登革热病毒和活的减毒嵌合登革热病毒。

40. 权利要求 39 的组合物，其中所述血清型 1、3 和 4 的登革热抗原各自为活的减毒嵌合登革热病毒，其中受体黄病毒的遗传骨架通过编码受体黄病毒 prM 和 E 蛋白的序列用登革热病毒的相应序列交换而被修饰。

41. 权利要求 40 的组合物，其中所述受体黄病毒是黄热病病毒。

42. 权利要求 38-41 中任一项的组合物，其中所述血清型 1、3 和 4 的登革热抗原各自为活的减毒嵌合登革热病毒，且所述血清型 2 的登革热抗原是包含与 SEQ ID NO: 24 具有至少 90% 序列同一性的核酸序列的血清型 2 的活的减毒登革热病毒。

43. 一种药物制剂，其包含前述权利要求中任一项的组合物和药学上可接受的载体、稀释剂或赋形剂。

44. 用于疗法的权利要求 1-42 中任一项的组合物。

45. 用于保护人类受试者免于由血清型 2 的登革热病毒引起的登革热病的方法的权利要求 1-42 中任一项的组合物。

46. 权利要求 38-42 中任一项的组合物，其用于保护人类受试者免于由血清型 1、血清型 2、血清型 3 或血清型 4 的登革热病毒引起的登革热病的方法。

47. 一种保护人类受试者免于由血清型 2 的登革热病毒引起的登革热病的方法，其中所述方法包括给予所述受试者有效量的权利要求 1-42 中任一项的组合物。

48. 一种保护人类受试者免于由血清型 1、血清型 2、血清型 3 或血清型 4 的登革热病毒引起的登革热病的方法，其中所述方法包括给予所述受试者有效量的权利要求 38-42 中任一项的组合物。

49. 一种药盒，所述药盒包含权利要求 1-42 中任一项的组合物和所述组合物在保护人类受试者免于由血清型 2 的登革热病毒引起的登革热病的方法中的使用说明书。

50. 一种药盒，所述药盒包含权利要求 38-42 中任一项的组合物和所述组合物在保护

人类受试者免于由血清型 1、血清型 2、血清型 3 或血清型 4 的登革热病毒引起的登革热病的方法中的使用说明书。

用于防止登革热病毒感染的疫苗组合物

发明领域

[0001] 本发明涉及疫苗组合物和所述组合物在保护人类受试者免于登革热病的方法中的用途。

[0002] 发明背景

登革热是继疟疾之后的第二最重要的感染性热带病，世界人口中大约有一半生活在有流行病传播风险的地区。估计每年有 5 千万 -1 亿例登革热病，导致 500,000 名患者因登革出血热 (DHF) 住院，并导致约 25,000 例死亡。

[0003] 登革热病感染流行于 100 多个热带国家，这些国家中有 60 个已证实有登革出血热 (DHF) (Gubler, 2002, TRENDS in Microbiology, 10: 100-103)。

[0004] 登革热病是由黄病毒属的 4 种在抗原上截然不同但密切相关的登革热病毒血清型引起的 (Gubler 等, 1988, 载于 : Epidemiology of arthropod-borne viral disease. Monath TPM 编辑 , Boca Raton (FL): CRC Press: 223-60 ;Kautner 等 , 1997, J. of Pediatrics, 131: 516-524 ;Rigau-Perez 等, 1998, Lancet, 352: 971-977 ;Vaughn 等, 1997, J. Infect. Dis., 176: 322-30)。

[0005] 登革热病通常通过被登革热病毒感染的埃及伊蚊 (*Aedes aegypti*) 吸血时注入该病毒而传播。在 4-10 天的潜伏期后，疾病突然开始，并接着以下 3 个阶段 :发热 (2-7 天)、危急 (24-48 小时—期间可能出现严重的并发症) 和恢复 (48-72 小时)。在危急期，可能出现危及生命的并发症，例如出血、休克和急性器官损害。适当管理这些不可预测的结局可降低病死率。在 7-10 天之后完成登革热的医治，但是长期虚弱是正常的。通常观察到白细胞和血小板数减少。

[0006] 登革出血热 (DHF) 是登革热病毒感染的潜在致死并发症。除有极度嗜睡和困倦以外，DHF 的特征还在于高烧和登革热病的症状。血管通透性增加和稳态异常可导致血容量降低、低血压，以及在严重情况下低血容量性休克和内出血。两个因素在 DHF 的发生中似乎起主要作用—快速病毒复制伴高水平的病毒血症 (该病的严重程度与病毒血症水平有关 ;Vaughn 等 , 2000, J. Inf. Dis., 181: 2-9) 和主要炎性反应伴高水平炎性介质释放 (Rothman 和 Ennis, 1999, Virology, 257: 1-6 ;Alan L. Rothman. 2011, Nature Reviews Immunology, 11: 532-543)。在无治疗的情况下，DHF 的死亡率可达 10%，但在可获得治疗的大多数中心区，死亡率 < 1%。

[0007] 登革热休克综合征 (DSS) 是 DHF 的常见进展，并且常常是致死的。DSS 产生于导致血浆渗漏进入血管外腔隙的泛发性血管炎。DSS 的特征是脉量快且差、低血压、四肢发冷和烦燥。

[0008] 在亚洲，主要在儿童中观察到 DHF 和 DSS，患有 DHF 儿童中约 90% 小于 15 岁 (Malavige 等 , 2004, Postgrad Med. J., 80: 588-601 ;Meulen 等 , 2000, Trop. Med. Int. Health, 5:325-9)。相比之下，在加勒比海和中美洲的爆发主要累及成人 (Malavige 等 , 2004, Postgrad Med. J., 80: 588-601)。

[0009] 登革热病毒的 4 种血清型具有约 60-80% 序列同源性。被一种登革热血清型感

染提供持久的同源免疫但有限的异源免疫 (Sabin, 1952, Am. J. Trop. Med. Hyg., 1: 30-50)。因此,被一种登革热血清型感染的个体之后可能被不同的血清型感染。在过去,曾认为产生于不同登革热病毒血清型的第二次感染在理论上是发生 DHF 的风险因素,因为显示 DHF 的大部分患者之前曾暴露于其它 4 种登革热病毒血清型的至少一种。

[0010] 至今,没有针对登革热病的特效治疗。对登革热病的治疗是针对症状的,卧床休息,通过退热药和镇痛药控制发烧和疼痛及大量饮水。DHF 的治疗需要平衡液体丢失、置换凝血因子和输注肝素。

[0011] 由于登革热预防措施 (例如蚊虫控制和个人防护免叮咬) 的功效有限,难于实施而且昂贵,因此安全和有效的登革热疫苗可能是最佳的预防方式。然而,没有目前可获得的经许可的该类型的疫苗。

[0012] 因此需要开发当用于保护人类受试者免于登革热病的方法时证实是有功效的疫苗组合物。

[0013] 发明概述

本发明涉及登革热病毒血清型 2 疫苗组合物,其包含 :

(i) 选自以下的登革热抗原 :

(a) 活的减毒登革热病毒 ;

(b) 灭活的登革热病毒 ;

(c) 活的减毒或灭活的嵌合登革热病毒 ;

(d) 登革热病毒样颗粒 (VLP) ;和

(e) (a)-(d) 的两种或更多种的组合 ;

或

(ii) 能够在人细胞中表达登革热抗原的核酸构建体或病毒载体,所述登革热抗原是登革热 VLP ;

其中所述登革热抗原包含与 SEQ ID NO: 12 具有至少 90% 同一性的多肽。

[0014] 本发明还涉及包含选自以下血清型 2 的登革热抗原的疫苗组合物 :(a) 活的减毒登革热病毒 ;(b) 灭活的登革热病毒 ;(c) 活的减毒或灭活的嵌合登革热病毒 ;或 (d) (a)-(c) 的两种或更多种的组合 ;其中所述登革热抗原包含编码包含权利要求中限定的一种或多种多肽的蛋白质的核苷酸序列。

[0015] 包含选自以下血清型 2 的登革热抗原的疫苗组合物 :(a) 活的减毒登革热病毒 ;(b) 灭活的登革热病毒 ;(c) 活的减毒或灭活的嵌合登革热病毒 ;或 (d) (a)-(c) 的两种或更多种的组合 ;其中所述登革热抗原包含与选自以下的序列具有至少 90% 序列同一性的核苷酸序列 :SEQ ID NO: 1 的 RNA 等同物、SEQ ID NO: 4 的 RNA 等同物、SEQ ID NO: 5 的 RNA 等同物、SEQ ID NO: 6 的 RNA 等同物、SEQ ID NO: 7 的 RNA 等同物和 SEQ ID NO: 25。

[0016] 本发明还涉及包含本发明的疫苗组合物和药学上可接受的载体、稀释剂或赋形剂的药物制剂。

[0017] 本发明还涉及用于治疗的本发明的疫苗组合物。

[0018] 本发明还涉及用于保护人类受试者免于由血清型 2 的登革热病毒引起的登革热病的方法的本发明的疫苗组合物。

[0019] 本发明还涉及本用于产生针对血清型 2 的登革热病毒的中和抗体的方法的本发

明的疫苗组合物。

[0020] 本发明还涉及本发明的疫苗组合物，其包含用于产生针对登革热的 4 种血清型的中和抗体的方法的血清型 1 的登革热抗原、血清型 2 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原。

[0021] 本发明还涉及本发明的疫苗组合物在用于制备保护人类受试者免于由血清型 2 的登革热病毒引起的登革热病的药物中的用途。

[0022] 本发明还涉及保护人类受试者免于由血清型 2 的登革热病毒引起的登革热病的方法，其中所述方法包括给予所述受试者有效量的本发明的组合物。

[0023] 本发明还涉及包括本发明的组合物和所述组合物在保护人类受试者免于由血清型 2 的登革热病毒引起的登革热病的方法中的使用说明书的药盒。

[0024] 本发明涉及用于保护人类受试者免于登革热病的方法的疫苗组合物，其中所述组合物包含：

(i) 选自以下的登革热抗原：

(a) 活的减毒登革热病毒；

(b) 灭活的登革热病毒；

(c) 活的减毒或灭活的嵌合登革热病毒；

(d) 登革热病毒样颗粒 (VLP)；和

(e) (a)-(d) 的两种或更多种的组合；

或

(iii) 能够在人细胞中表达登革热抗原的核酸构建体或病毒载体，所述登革热抗原是登革热 VLP。

[0025] 本发明还涉及本发明的疫苗组合物在用于制备保护人类受试者免于登革热病的药物中的用途。

[0026] 本发明还涉及保护人类受试者免于登革热病的方法，其中所述方法包括给予所述人类受试者有效量的本发明的组合物。

[0027] 另外，本发明涉及包括本发明的组合物和在保护人类受试者免于登革热病的方法中使用所述组合物的使用说明书的药盒。

[0028] 附图描述

图 1说明 YF-VAX cDNA 通过 RT-PCR 构建和克隆。

[0029] 定义

本文所用术语“登革热病”是指在被 4 种登革热病毒血清型的任何一种感染之后个体出现的临床症状。自 1970 以来，按照世界卫生组织准则，临床登革热被归类为：(i) 登革热 (dengue fever)，或 (ii) 登革出血热 (World Health Organization. Dengue hemorrhagic fever: Diagnosis, treatment, prevention and control 第2版. Geneva: WHO, 1997；ISBN 92 4 154500 3)。2009 年，WHO 颁布了新的准则，将临床登革热归类为：(i) 发出警告标志或无警告标志的登革热，或 (ii) 严重登革热。两种分类见 Srikiatkachorn 等，Clin. Infect. Dis. (2011) 53(6): 563 的图 1 和 2。按照较早的分类，登革热的特征为选自以下的至少两种症状：头痛、关节痛、眶后痛、皮疹、肌痛、出血现象和白细胞减少以及支持性血清学或在与其它已证实的登革热病情的相同地点和时间出现过。当发热、出血现象、血小

板减少和血浆渗漏的证据所有这些全被观察到时，证实已发展成为登革出血热。按照较新的分类，登革热的诊断要求存在发热和选自恶心、呕吐、皮疹、疼痛的至少两种临床症状、阳性止血带试验或选自以下的任何警告标志：腹部疼痛和触痛、持续呕吐、临床流体蓄积、粘膜出血、嗜眠或烦燥、肝增大超过 2 cm 或血细胞比容增加同时伴血小板计数快速降低。当观察到以下任何事件时，诊断为重度登革热：导致休克或呼吸窘迫的严重血浆渗漏、由临床医生评价为严重出血或严重器官受累。

[0030] 本文所用术语“登革出血热或 DHF”是指病毒学上已证实的登革热病，其中发热、出血现象、血小板减少和血浆渗漏的证据全都被观察到。还根据其严重程度可进一步定义本文所用的 DHF。例如，DHF 可定义为 I 级、II 级、III 级或 IV 级 (World Health Organization. Dengue hemorrhagic fever: Diagnosis, treatment, prevention and control 第 2 版. Geneva: WHO, 1997；ISBN 92 4 154500 3)。I 级定义为发热伴发非特异性全身症状；唯一的出血现象是阳性止血带试验和 / 或易瘀伤。II 级定义为除 I 级患者的表现以外的自发性出血，通常呈皮肤或其它出血形式。III 级定义为表现为脉搏急速微弱和脉压缩小或低血压的循环衰竭，伴以存在皮肤湿冷和烦燥。IV 级定义为无可检测血压或脉搏的深度休克。本领域技术人员应了解，在本发明的实践中，例如防止 DHF 的方法，所述 DHF 不必是病毒学上已证实的。

[0031] 本文所用术语“病毒学上已证实的登革热”是指由通过例如反转录酶聚合酶链式反应 (RT-PCR) 或登革热非结构 1 (NS1) 蛋白酶联免疫吸附测定法 (ELISA) 证实的登革热病毒诱导的急性发热发作。在 RT-PCR 方法中，血清样品通过 Callahan 等人的方法测试 (J. Clin. Microbiol. (2001) 39: 4119)。简单地说，使用商用试剂盒，从血清中提取 RNA 以弃去潜在的 Taq 聚合酶抑制剂或干扰因子。然后用来自登革热 NS5 基因序列的血清型特异性引物进行 RT-PCR 反应。结果表示为与含有已知浓度的整合至质粒的病毒基因组血清型特异性核酸序列的标准品比较的 $\log_{10}$GEQ（基因组当量）/mL 的浓度。在 ELISA 方法中，将 50 μL 的患者血清、阳性对照、阴性对照或截止值对照 (cut-off control) 1:2 稀释于样品稀释剂中，并与 100 μL 稀的辣根过氧化物酶 (HRP) 标记的抗 NS1 单克隆 Ab (MAb) 混合。加入稀的血清和缀合物以俘获抗 NS1 MAb 包被的微孔，将板在 37℃下孵育 90 分钟。当 NS1 存在于血清中时，形成俘获 MAb/NS1/HRP 标记的 MAb 复合物。通过阳性孔中的比色反应检测复合物，所述比色反应通过加入 160 μL 3,3’,5,5’ 四甲基联苯胺 (TMB) 底物并在室温下避光孵育 30 分钟来诱导。加入 100 μL 终止溶液 (1N H_2SO_4) 终止反应，并读板。通过试验样品的平均光密度 (OD) 除以截止值对照的平均 OD（一式四份测试），求出各样品的样品比率。样品比率 <0.5、0.5-<1.0 和≥ 1 分别表示阴性、不明确和阳性结果。

[0032] 本文所用术语“病毒学上已证实的重度登革热”是指如 1997 WHO 分类定义的登革出血热 (DHF)，且其进一步的特征为下列另外列举的症状：需要输血的出血、毛细血管通透性的客观证据、循环衰竭的征兆或脏象。

[0033] 本文所用术语“登革热休克综合征”是指上文定义的最严重的 DHF 并发症。按照 1997 WHO 分类，DSS 相当于 III 级和 IV 级的 DHF。

[0034] 术语“登革热病毒”、“登革热病毒”和“DEN”可互换使用。它们是指属于黄病毒科 (flaviviridae) 黄病毒属的正单链 RNA 病毒。有 4 种不同血清型的登革热病毒（血清型 1、2、3 和 4），它们具有约 60-80% 序列同源性。基因组的组构包含下列元件：5’ 非编码区

(NCR)、编码结构蛋白(衣壳(C)、前膜(prM)和包膜(E))的区域和编码非结构蛋白(NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5)的区域和3’ NCR。登革热病毒基因组编码不间断编码区,其翻译为进行翻译后加工的单一多蛋白。prM-E序列中所包括的亚序列可以不同方式编号:(i)总的prM-E蛋白序列从1位到661位编号,其preM蛋白序列指定为1位到90/91位,M蛋白序列指定为91/92位-166位,E蛋白序列指定为167位-661位;(ii) prM和M蛋白序列在一起编号,即从总序列的1位-166位,E单独从1位-495位编号;(iii) prM、M和E序列分别编号,即prM从1位-90/91位编号,M从1位到75/76位编号,E从1位到495位编号。在本公开内容中,E蛋白总是从1位-495位编号。例如,本文命名为E-154的残基是指E蛋白的154位。

[0035] 在本发明的情况下,“疫苗登革热病毒”是指通过将疫苗登革热病毒给予免疫活性受试者能够诱导针对所述疫苗登革热病毒来自于其中的登革热病毒血清型的中和抗体的病毒。可用于本发明方法的疫苗登革热病毒的实例包括灭活登革热病毒、活的减毒登革热病毒和活的减毒或灭活嵌合登革热病毒。用于本发明的疫苗登革热病毒的血清型包括血清型1、2、3和4。优选用于本发明的疫苗登革热病毒是活的减毒嵌合登革热病毒。

[0036] 本文所用表述“灭活病毒”是指在允许复制相应的野生型病毒的细胞中不能复制到任何有效程度的病毒。病毒可通过本领域技术人员熟知的多种方法灭活。用于灭活病毒的方法的实例包括化学处理或照射处理(包括热或通常呈X射线或紫外辐射形式的电磁辐射)。

[0037] 本文所用的术语“灭活登革热病毒”是指含有全部登革热结构蛋白(env、前膜/膜蛋白和衣壳蛋白)和灭活病毒RNA的灭活野生型病毒。灭活的登革热病毒也可指灭活的嵌合登革热病毒。灭活登革热病毒描述于例如美国专利号6,254,873。

[0038] 本文所用术语“活的减毒病毒或LAV”是指不能诱导特征在于与相应的野生型病毒相关的相同组症状的疾病状态的病毒。活的减毒病毒的实例是本领域众所周知的。可通过例如重组DNA技术、位点定向诱变、遗传操作、复制型细胞的系列传代、化学诱变处理或电磁辐射,自野生型病毒制备活的减毒病毒。

[0039] 本文所用术语“活的减毒登革热病毒”是指通过导致毒力减弱和无法诱导特征在于与相应的野生型登革热病毒有关的相同组症状的疾病状态的遗传修饰,来源于有毒的野生型登革热病毒的活的登革热病毒。可用于本发明实践中的活的减毒登革热病毒的实例包括VDV-1、VDV-2和描述于例如以下申请的毒株:WO 02/66621、WO 00/57904、WO 00/57908、WO 00/57909、WO 00/57910、WO 02/0950075和WO 02/102828。可用于本发明方法的血清型1的活的减毒登革热病毒包括VDV-1。可用于本发明方法的血清型2的活的减毒登革热病毒包括VDV-2和LAV-2。

[0040] “VDV”和“Vero登革热疫苗”在本文中可互换使用,并命名为能够在Vero细胞中复制并能够在人中诱导特异性体液反应(包括诱导中和抗体)的活的减毒登革热病毒。

[0041] DEN-1 16007/PDK13毒株,亦称“LAV1”,来源于通过原代狗肾(PDK)细胞进行11次传代的野生型DEN-1(登革热病毒血清型1) 16007毒株(DEN-1 16007/PDK11)。LAV1描述于Mahidol University名下的专利申请EP1 159968,并提交给国家微生物培养物保藏中心(National Microorganisms Cultures Collection,CNCM),编号为I-2480。“VDV-1”是通过对Vero细胞的后续适应而来源于LAV1的病毒;在这一方面,从LAV1提取RNA并纯

化后，转染至 Vero 细胞。随后通过板纯化，在 Vero 细胞中扩增，获得 VDV-1 毒株。与 DEN-1 16007/PDK13 毒株（通过 PDK 细胞 13 次传代）相比，VDV-1 毒株具有 14 个另外的突变。用于制备和表征 VDV-1 毒株的方法描述于 Sanofi-Pasteur 和疾病控制与预防中心 (Center for Disease Control and Prevention) 名下的以编号 WO06/134433 提交的国际专利申请。

[0042] DEN-2 16681/PDK53 毒株，亦称为“LAV2”，获自野生型毒株 DEN-2（登革热病毒血清型 2）16681，其通过 PDK 细胞进行 50 次传代 (DEN-2 16681/PDK50)。LAV2 描述于 Mahidol University 名下的专利申请 EP1159968，并提交给国家微生物培养物保藏中心 (CNCM)，编号为 I-2481。“VDV-2”是通过对 Vero 细胞的后续适应而来源于 LAV2 的毒株；在这一方面，自 LAV2 提取 RNA 并纯化后，转染至 Vero 细胞。随后通过板纯化和在 Vero 细胞中扩增来获得 VDV-2 毒株。与 DEN-2 16681/PDK53 毒株（通过 PDK 细胞 53 次传代）相比，VDV-2 毒株具有 10 个另外的突变，包括 4 个沉默突变。用于制备和表征 VDV-2 毒株的方法描述于以 Sanofi-Pasteur 和疾病控制与预防中心 (Center for Disease Control and Prevention) 的名义以编号 WO06/134443 提交的国际专利申请。VDV-2 毒株的完整核酸序列如 SEQ ID NO: 24 中所示。VDV-2 毒株的 E 蛋白的序列如 SEQ ID NO: 26 所示，VDV-2 毒株 M 蛋白的序列如 SEQ ID NO: 27 所示。

[0043] 通过在 Vero 细胞中扩增制备 VDV 1 和 2 毒株。收获所产生的病毒，并通过过滤从细胞碎片中澄清。通过用酶处理消化 DNA。通过超滤清除杂质。可通过浓缩方法提高感染滴度。在加入稳定剂后，毒株在用前以冻干或冷冻形式保存，然后在需要时重构。

[0044] 在本发明的情况下，“登革热嵌合体或嵌合登革热病毒”意指这样的受体黄病毒，其中遗传骨架通过编码受体黄病毒的 prM 和 E 蛋白的序列用登革热病毒的相应序列交换而被修饰。受体黄病毒通常可被减毒。受体黄病毒可以是黄热病 (YF) 病毒，例如减毒的 YF 17D、YF 17DD 和 YF 17D204 (YF-VAX®) 病毒；如果这样的话，所述嵌合体被称为 YF/ 登革热嵌合体。受体黄病毒还可以是登革热病毒，如果这样的话，它被称为登革热 / 登革热嵌合体，特征为 prM 和 E 蛋白的登革热病毒血清型与特征为遗传骨架的受体登革热病毒血清型相同或不同。当血清型是相同的时，受体登革热病毒和 prM 和 E 蛋白编码序列来源于其中的登革热病毒是相同血清型的两种不同的病毒毒株。为了用于本发明，嵌合登革热病毒通常是 YF/ 登革热嵌合体。嵌合登革热病毒优选是灭活或活的减毒嵌合登革热病毒。有利的是，本发明的活的减毒嵌合登革热病毒的受体黄病毒是 YF 17D 或 YF 17D204 (YF-VAX®)。按照一个实施方案，登革热嵌合体是灭活病毒。按照备选的实施方案，登革热嵌合体是活的减毒病毒。可用于本发明的疫苗组合物的登革热嵌合体包括 Chimerivax™登革热血清型 1（亦称为 CYD-1）、Chimerivax™登革热血清型 2（亦称为 CYD-2）、Chimerivax™登革热血清型 3（亦称为 CYD-3）和 Chimerivax™登革热血清型 4（亦称为 CYD-4）。

[0045] 可用于本发明的实践中的嵌合登革热病毒的实例包括描述于专利申请 WO 98/37911 的登革热 /YF 嵌合病毒和登革热 / 登革热嵌合体，例如描述于专利申请 WO 96/40933 和 WO 01/60847 的那些。

[0046] 在一个实施方案中，嵌合 YF/ 登革热病毒包含减毒黄热病病毒毒株 YF17D 的基因组骨架 (Theiler M. 和 Smith H.H., 1937, J.Exp.Med., 65. 767-786)，例如病毒 YF17D/DEN-1、YF17D/DEN-2、YF17D/DEN-3 和 YF17D/DEN-4。可以使用的 YF17D 毒株的实例包括 YF17D204 (YF-VAX(R), Sanofi-Pasteur, Swiftwater, PA, USA；Stamaril(R),

Sanofi-Pasteur, Marcy I'Etoile, France;ARILVAX(TM), Chiron, Speke, Liverpool, UK;FLAVIMUN(R), Berna Biotech, Bern, Switzerland;YF17D-204 France (X15067, X15062);YF17D-204,234 US (Rice 等, 1985, Science, 229:726-733) 或相关毒株 YF17DD (Genbank 登录号 U17066)、YF17D-213 (Genbank 登录号 U17067) 和 Galler 等人描述的毒株 YF17DD (1998, Vaccines, 16(9/10): 1024-1028)。在另一个实施方案中,嵌合 YF/ 登革热病毒包含减毒黄热病病毒毒株 YF17D204 (YF-VAX®) 的基因组骨架。

[0047] 特别适用于本发明实践中的嵌合登革热病毒的一个实例是"Chimerivax登革热病毒"。本文所用"Chimerivax 登革热病毒"是活的减毒嵌合 YF/ 登革热病毒,其包含 YF17D 或 YF17D204 (YF-VAX®) 病毒的基因组骨架,其中编码前膜 (prM) 和包膜 (E) 蛋白的核酸序列被编码登革热病毒的相应结构蛋白的核酸序列置换。用于本发明的优选的嵌合登革热病毒是活的减毒嵌合 YF/ 登革热病毒,其包含 YF17D 病毒的基因组骨架,其中编码前膜 (prM) 和包膜 (E) 蛋白的核酸序列被编码登革热病毒的相应结构蛋白的核酸序列置换。用于本发明的优选的嵌合登革热病毒是活的减毒嵌合 YF/ 登革热病毒,其包含 YF17D204 (YF-VAX®) 病毒的基因组骨架,其中编码前膜 (prM) 和包膜 (E) 蛋白的核酸序列被编码登革热病毒的相应结构蛋白的核酸序列置换。所述 Chimerivax 病毒的构建可按照或基本按照 Chambers 等 (1999, J.Virology 73(4):3095-3101) 的教导实现。通过使用来自毒株 DEN 1 PUO359 (TYP1 140)、DEN2 PUO218、DEN3 PaH881/88 和 DEN 4 1228 (TVP 980) 的 prM 和 E 序列和 YF17D 病毒的基因组骨架,产生描述于实施例中的具体 Chimerivax (CYD) 登革热病毒。所述特殊的 Chimerivax 毒株在本文 (参见本发明实施例) 分别称为"CYD-1"、"CYD-2"、"CYD-3"和"CYD-4"。这些特殊的 CYD-1、CYD-2、CYD-3 和 CYD-4 毒株的制备详细描述于国际专利申请 WO 98/37911、WO 03/101397、WO 07/021672、WO 08/007021、WO 08/047023 和 WO 08/065315,可引用其制备方法的精确描述。或者,其它登革热病毒毒株可用作核酸来源以便于可用于本发明实践的嵌合病毒的构建,例如用于其它 Chimerivax 登革热血清型 1 (CYD-1)、Chimerivax 登革热血清型 2 (CYD-2)、Chimerivax 登革热血清型 3 (CYD-3) 和 Chimerivax 登革热血清型 4 (CYD-4) 毒株的构建中。有利的是,本发明的疫苗组合物,例如血清型 2 的嵌合登革热病毒可包含与来自实施例中描述的血清型 2 毒株 LAV-2、BID-V585、PR/DBO23 或 MD1280 的 prM-E 序列有至少 90%、至少 95%、至少 98% 或至少 99% 同一性的 prM-E 序列或可包含与 SEQ ID NO: 2 所示 prM-E 序列有至少 90%、至少 95%、至少 98% 或至少 99% 同一性的 prM-E 序列。有利的是,疫苗组合物,例如用于本发明方法的血清型 2 的嵌合登革热病毒可包含来自血清型 2 毒株 LAV-2、BID-V585、PR/DBO23 或 MD1280 的 prM-E 序列或来自实施例中描述的 SEQ ID NO: 2 的 prM-E 序列。当所述嵌合登革热病毒的受体基因组骨架来源于 YF-VAX® 时,所述毒株在本文被称为 CYD-LAV、CYD-BID、CYD-PR 和 CYD-MD。在本发明的疫苗组合物中,包含使用血清型 2 毒株 LAV-2 (SEQ ID NO: 8)、BID-V585 (SEQ ID NO: 9)、PR/DBO23 (SEQ ID NO: 10)、MD1280 (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列产生的或使用与来自血清型 2 毒株 LAV-2、BID-V585、PR/DBO23、MD1280 的 prM-E 序列或来自 SEQ ID NO: 2 的 prM-E 序列具有至少 90%、至少 95%、至少 98% 或至少 99% 同一性的 prM-E 序列产生的血清型 2 的嵌合登革热病毒的本发明的疫苗组合物可有利地与 CYD-1、CYD-3 和 CYD-4 联用。分别使用血清型 2 毒株 LAV-2 (SEQ ID NO: 8)、PR/DBO23 (SEQ ID NO: 10) 和 MD1280 (SEQ ID NO: 11) 的 prM-E 序列产生的血清型 2 的嵌合登革热病毒的实

例包括 CYD-LAV、CYD-PR 和 CYD-MD。

[0048] 可用于本发明的保护方法的嵌合登革热病毒的备选实施方案是这样的受体黄病毒，其中遗传骨架通过 (i) 编码受体黄病毒 E 蛋白的序列用登革热病毒的相应序列交换，和 (ii) 编码受体黄病毒 prM 蛋白的序列用非登革热黄病毒（例如 JEV 病毒）的相应序列交换而被修饰。通常，所述嵌合病毒可以是活的减毒病毒或灭活病毒。所述嵌合登革热病毒的实例描述于 WO2011/138586。

[0049] 用于本发明的疫苗组合物中的血清型 1 的疫苗登革热病毒可以是例如毒株 VDV1、CYD-1 或包含 DEN-1 16007/PDK13 毒株的 prM 和 E 氨基酸序列的 YF17D/DEN-1 嵌合病毒。用于本发明方法的血清型 2 的疫苗登革热病毒可以是例如毒株 VDV2、CYD-2、包含 DEN-2 16681/PDK53 毒株的 prM 和 E 氨基酸序列的 YF17D/DEN-2 嵌合病毒、包含 DEN-2 毒株 LAV-2、BID-V585、PR/DB023 或 MD1280 的 prM 和 E 氨基酸序列的嵌合病毒或包含与来自血清型 2 毒株 LAV-2、BID-V585、PR/DB023 或 MD1280 的 prM-E 序列具有至少 90%、至少 95%、至少 98% 或至少 99% 同一性或与 SEQ ID NO: 2 的 prM-E 序列具有至少 90%、至少 95%、至少 98% 或至少 99% 同一性的 prM-E 序列的嵌合病毒。用于本发明方法的血清型 3 的疫苗登革热病毒可以是例如 CYD-3 或备选的 YF17D/DEN-3 嵌合病毒。血清型 4 的疫苗登革热病毒的实例是 CYD-4 或备选的 YF17D/DEN-4 嵌合病毒。

[0050] 本发明的组合物包含至少一种登革热抗原。本发明的组合物通常包含登革热抗原，例如血清型 1、2、3 和 4 每一种的疫苗登革热病毒。登革热抗原，例如每种血清型的本发明的疫苗登革热病毒可如本发明描述的一样。例如，本发明的组合物可有利地包含登革热抗原的下列组合的任一种 :i) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-LAV 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 氨基酸序列的嵌合登革热病毒和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ;ii) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-BID 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ;(iii) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-PR 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ;(iv) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-MD 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原。例如，本发明的组合物还可有利地包含登革热抗原的下列组合的任一种 :i) CYD-1、CYD-LAV、CYD-3 和 CYD-4 ;ii) CYD-1、CYD-BID、CYD-3 和 CYD-4 ;(iii) CYD-1、CYD-PR、CYD-3 和 CYD-4 或 (iv) CYD-1、CYD-MD、CYD-3 和 CYD-4。本发明的组合物还可有利地包含登革热抗原的下列组合 :i) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、VDV2、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原。例如，本发明的组合物可有利地包含 CYD-1、VDV-2、CYD-3 和 CYD-4。如本文所述，本发明的组合物可有利地包含血清型 2 的登革热抗原，其包含 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列。如本文所述，本发明的组合物可有利地包含血清型 2 的登革热抗原，其包含与 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列具有至少 90% 同一性的序列。例如，所述序列可与 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID

NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列有至少 91%、至少 92%、至少 93%、至少 94%、至少 95%、至少 96%、至少 97%、至少 98% 或至少 99% 同一性。

[0051] 本文所用术语“病毒样颗粒或 VLP”是指不含复制性遗传物质但在其表面上以类似于病毒体结构的重复有序的阵列存在登革热 E 蛋白的病毒颗粒。通常，登革热 VLP 还含有登革热 prM 和 / 或 M 和 E 蛋白。VLP 可体外产生 (Zhang 等， J. Virol. (2011) 30 (8):333)。VLP 还可体内产生。为此目的，可通过本领域已知方法，例如通过使用病毒载体，将编码 prM 和 E 登革热蛋白质的核酸构建体（例如 DNA 或 RNA 构建体）导入受试者（例如人类受试者）的细胞中。可使用任何病毒载体，只要它能够含有和表达 prM 和 E 登革热病毒序列两者。可用于本发明方法的病毒载体的非限制性实例包括痘病毒（例如减毒 pox Ankara 病毒）和麻疹病毒。为了用于本发明，体内表达 VLP 的病毒载体的具体类别包括例如按照 Replivax™技术的复制缺陷型假性感染性 (PIV) 病毒 (Rumyantsev AA, 等， Vaccine. 2011 Jul 18;29(32):5184-94)。

[0052] 本文所用术语“复制缺陷型假性感染性病毒”是指由于在基因组中缺乏复制周期的必需序列，例如编码衣壳蛋白的序列，因此是体内复制缺陷型的病毒体颗粒。然而，病毒体颗粒可在提供反式必需序列的辅助细胞的培养物中繁殖。用于本发明的复制缺陷型假性感染性病毒包括能够表达任何血清型的登革热病毒的 prM 和 E 蛋白的上述定义的任何病毒。实例包括复制缺陷型黄病毒 / 登革热嵌合体，例如复制缺陷型西尼罗病毒 / 登革热、日本脑炎病毒 / 登革热和 YF/ 登革热嵌合体。

[0053] 可通过例如测量针对包含在组合物内的登革热病毒血清型引起的中和抗体滴度，来评价本发明的疫苗组合物在受试者中引起免疫应答（即诱导中和抗体产生）的能力。中和抗体滴度可通过蚀斑减少中和试验 (plaque-reduction neutralization test，$PRNT_{50}$) 测量。简单地说，在给予本发明的疫苗组合物后至少 28 天，在从接种受试者中收集的血清中测量中和抗体滴度。适当时，将血清（之前已热灭活）的 2 倍系列稀释液与恒定攻击剂量的血清型 1、2、3 或 4 的各登革热病毒（表示为 PFU/mL）混合。将混合物接种在具有汇合的 Vero 细胞单层的微量板的孔中。在吸附后，使细胞单层孵育几天。通过感染灶的形成表明登革热病毒感染的细胞的存在，因此可检测由于血清样品中中和抗体的存在所引起的病毒感染性的降低。所报告的值（终点中和滴度）表示当与阴性对照孔（其代表 100% 病毒载量）中的平均病毒灶计数相比时，登革热攻击病毒（在病毒灶计数中）≥ 50% 被中和的血清的最高稀释度。终点中和滴度以连续值提供。该测定法的定量下限 (LLOQ) 为 10 (1/dil)。一般认为，当滴度优于或等于 10 (1/dil) 时发生血清转化。由于 PRNT 试验可能在实验室间略有不同，因此 LLOQ 也可能略有不同。因此，通常认为，当滴度优于或等于试验的 LLOQ 时，发生血清转化。在下列参考文献中考虑了中和抗体滴度，但是作者没有建立保护的相关性 (Guirakhoo 等， J. Virol. (2004) 78 (9): 4761 ;Libraty 等， PLoS Medicine (2009) 6 (10) ;Gunther 等， Vaccine (2011) 29: 3895 和 Endy 等， J. Infect. Dis. (2004), 189(6): 990-1000)。

[0054] 术语“$CCID_{50}$”是指感染 50% 细胞培养物的病毒（例如疫苗病毒）的量。$CCID_{50}$ 测定法是含统计滴度计算的有限稀释测定法 *(Morrison D等， J Infect Dis. 2010; 201(3):370-7)*。

[0055] 术语“人类受试者”意指不同年龄的男性和女性。优选本发明的人类受试者小于

18 岁或小于 12 岁。例如，本发明的人类受试者可为 0-17 岁、0-11 岁、4-17 岁、4-11 岁、4-6 岁、6-8 岁、8-10 岁、2-8 岁、2-11 岁、2-14 岁、9-16 岁、12-17 岁或 18-45 岁。更优选本发明的人类受试者为 4-11 岁、2-14 岁或 9-16 岁。本发明的人类受试者可为至少 9 月龄或小于 9 月龄。例如本发明的人类受试者可为 9 个月 -16 岁、9 个月 -14 岁、9 个月 -11 岁或 9 个月 -8 岁。本发明的人类受试者可为至少 9 月龄，对本文定义的疫苗组合物的任何组分无严重变态反应史，无先天性或获得性免疫缺陷，无症状性 HIV 感染，且所述受试者应未怀孕或哺乳。

[0056] 本文所用表述“黄病毒幼稚受试者”是指未曾被黄病毒感染、之前也未用黄病毒疫苗免疫的受试者，即取自所述受试者的血清样品将在黄病毒 ELISA 或 PRNT 测定法中产生阴性结果。

[0057] 本文所用表述“登革热幼稚受试者”是指未曾被登革热病毒感染、之前也未用登革热疫苗免疫的受试者，即取自所述受试者的血清样品将在登革热 ELISA 或 PRNT 测定法中产生阴性结果。

[0058] 本文所用表述“黄病毒免疫受试者”是指在给予本发明的疫苗组合物之前被黄病毒感染或经免疫的受试者，即取自所述受试者的血清样品将在黄病毒 ELISA 或 PRNT 测定法中产生阳性结果。

[0059] 本文所用表述“登革热免疫受试者”是指在给予本发明的疫苗组合物之前被登革热病毒感染或经登革热疫苗免疫的受试者，即取自所述受试者的血清样品将在登革热 ELISA 或 PRNT 测定法中产生阳性结果。

[0060] 按照本发明，本文所用的“保护方法”导致在暴露于登革热病毒的人类受试者中发生登革热病的严重程度或可能性降低。有利的是，所述降低有统计显著性。例如，本发明的保护方法可导致本文定义的登革热病的至少一种症状减轻或这些症状的任何两种或更多种的组合的减轻。保护可导致以下的任一种或多种：

(i) 由任何血清型的登革热病毒引起的有症状的病毒学上已证实的登革热病的发生率或可能性统计显著性地降低，例如其预防；

(ii) 由血清型 1、3 或 4 的任一种的登革热病毒引起的有症状的病毒学上已证实的登革热病的发生率或可能性统计显著性地降低，例如其预防；

(iii) 由任何血清型的登革热病毒引起的有症状的登革热病的发生率或可能性统计显著性地降低，例如其预防；

(iv) 由血清型 1、3 或 4 的任一种的登革热病毒引起的有症状的登革热病的发生率或可能性统计显著性地降低，例如其预防；

(v) 由任何血清型的登革热病毒引起的病毒学上已证实的重度登革热的发生率或可能性统计显著性地降低，例如其预防；

(vi) 由任何血清型的登革热病毒引起的重度登革热病的发生率或可能性统计显著性地降低，例如其预防；

(vii) 由任何血清型的登革热病毒引起的 I-IV 级登革出血热病例的发生率或可能性统计显著性地降低，例如其预防；

(viii) 由任何血清型的登革热病毒引起的 I 级 DHF 病例的发生率或可能性统计显著性地降低，例如其预防；

(ix) 由任何血清型的登革热病毒引起的II级DHF病例的发生率或可能性统计显著性地降低,例如其预防;

(x) 由任何血清型的登革热病毒引起的III级DHF病例的发生率或可能性统计显著性地降低,例如其预防;

(xi) 由任何血清型的登革热病毒引起的IV级DHF病例的发生率或可能性统计显著性地降低,例如其预防;

(xii) 发热的发生率或可能性统计显著性地降低,例如其预防,或发热的平均持续时间缩短和/或强度减弱;

(xiii) 由血细胞比容的变化限定的血浆渗漏的发生率或可能性统计显著性地降低,例如其预防,或由血细胞比容的变化限定的血浆渗漏的平均值降低;

(xiv) 血小板减少的发生率或可能性统计显著性地降低,例如其预防,或血小板减少的平均值降低;

(xv) 包括丙氨酸氨基转移酶(ALT)和天冬氨酸氨基转移酶(AST)在内的肝酶水平升高的发生率或可能性统计显著性地降低,例如其预防;

(xvi) 因由任何血清型的登革热病毒引起的病毒学上已证实的登革热病所致住院的发生率或可能性统计显著性地降低,例如其防止;

(xvii) 因由任何血清型的登革热病毒引起的登革热病所致住院的发生率或可能性统计显著性地降低,例如其防止;

(xviii) 因病毒学上已证实的登革热病所致住院时间长度统计显著性地缩短;

(xix) 因登革热病所致株院时间长度统计显著性地缩短。

[0061] 按照标准住院规程监测并记录发热的持续时间和强度。在人类受试者中,发热(即发热发作)定义为在至少4小时间隔期间两次测量的至少37.5℃的2次温度读数的观察。血细胞比容、血小板减少和肝酶水平的测量是例如药典中所描述的本领域技术人员众所周知的标准试验。

[0062] 有关由具体的登革热病毒血清型引起的登革热病,可证实例如上述(i)-(xix)点定义的针对登革热病的保护。例如,有关由血清型1的登革热病毒、血清型2的登革热病毒、血清型3的登革热病毒或血清型4的登革热病毒引起的登革热病,可证实本文定义的针对登革热病的保护。有利的是,有关由例如血清型1或血清型3的登革热病毒、血清型1或血清型4的登革热病毒、血清型3或血清型4的登革热病毒、血清型1或血清型2的登革热病毒、血清型2或血清型3的登革热病毒、血清型2或血清型4的登革热病毒、血清型1、2或3的登革热病毒、血清型1、3或4的登革热病毒、血清型2、3或4的登革热病毒或血清型1、2、3或4的登革热病毒引起的登革热病,可证实本文定义的针对登革热病的保护。

[0063] 可在人类受试者的特定亚群中有利地证实本文定义的针对登革热病的保护。例如,可在小于18岁或小于12岁的人类受试者中有利地证实针对登革热病的保护。例如,本发明的人类受试者可为0-17岁、0-11岁、4-17岁、4-11岁、4-6岁、6-8岁、8-10岁、2-8岁、2-11岁、2-14岁、9-16岁、12-17岁或18-45岁。更优选本发明的人类受试者为4-11岁、2-14岁或9-16岁。本发明的人类受试者可为至少9月龄或小于9月龄。例如本发明的人类受试者可为9个月-16岁、9个月-14岁、9个月-11岁或9个月-8岁。本发明的人类受试者可为至少9月龄,对本文定义的疫苗组合物的任何组分无严重变态反应史,无先天性

或获得性免疫缺陷，无有症状的 HIV 感染，且所述受试者应未怀孕或哺乳。

[0064] 可在世界的特定国家、区域或地区有利地证实本文定义的针对登革热病的保护。例如，可在登革热病区有利地证实针对登革热病的保护。例如，其中保护可被证实的本发明的登革热病区可包含列入热带或亚热带的那些美洲国家或其部分。其中按照本发明保护可被证实的登革热病区因此可包括以下的任一个或多个：巴西、委内瑞拉、哥伦比亚、厄瓜多尔、秘鲁、玻利维亚、巴拉圭、巴拿马、哥斯达黎加、尼加拉瓜、洪都拉斯、萨尔瓦多、危地马拉、伯利兹、墨西哥、美国和加勒比海岛屿。在一个具体的实施方案中，其中保护可被证实的本发明的登革热病区可包括：巴西、哥伦比亚、洪都拉斯、墨西哥和波多黎各。其中按照本发明保护可被证实的登革热病区还可包括在热带和亚热带内的南亚和大洋洲国家。其中保护可被证实的本发明的登革热病区因此可包括以下的任一个或多个：印度、缅甸 (Myanmar/Burma)、泰国、老挝、越南、柬埔寨、印度尼西亚、马来西亚、新加坡、菲律宾、台湾、巴布亚新几内亚和澳大利亚。在其中按照本发明保护可被证实的登革热病区中，野生型登革热病毒的特定血清型、毒株或基因型可为主要的传播毒株。例如，血清型 2 的登革热病毒可称为具有亚洲 I 或亚洲 / 美洲基因型。亚洲 / 美洲基因型毒株的特征在于下列残基的至少 1 个、至少 2 个、至少 3 个、至少 4 个、至少 5 个或所有 6 个：分别在 prM-16、E-83、E-203、E-226、E-228 和 E-346 位上的 Arg、Asn、Asp、Thr、Gly 和 His（其中 prM-16 表示 prM 蛋白的第 16 位，E-83 表示 E 蛋白的第 83 位等）。亚洲 I 基因型毒株的特征在于下列残基的至少 1 个、至少 2 个、至少 3 个、至少 4 个、至少 5 个或所有 6 个：分别在 prM-16、E-83、E-203、E-226、E-228 和 E-346 位上的 Ile、Lys、Asn、Arg、Glu 和 Tyr（参见 Hang 等，PLoS NTD，4(7)：e757 的表 1)。其中按照本发明保护可被证实的优选的登革热病区是其中具有亚洲 / 美洲基因型的登革热病毒是主要的传播毒株的登革热病区，即在所述登革热病区中至少 50%、至少 60%、至少 70%、至少 80%、至少 90%、至少 95% 或 100% 登革热病病例由具有亚洲 / 美洲基因型的登革热病毒引起。其中按照本发明保护可被证实的优选的登革热病区是其中血清型 1、3 或 4 的任一种或多种的登革热病毒是主要传播血清型的登革热病区，即至少 50%、至少 60%、至少 70%、至少 80%、至少 90%、至少 95% 或 100% 的登革热病病例由血清型 1、3 或 4 的登革热病毒引起。

[0065] 本文所用的指定 DNA 序列的术语“RNA 等同物”是指其中脱氧胸苷被尿苷置换的序列。因为所述 DNA 序列构成登革热病毒的 cDNA 序列，因此等同的 RNA 序列构成这些登革热病毒的正链 RNA。

[0066] 若干实施方案的综述

本发明人首次证实了一种疫苗组合物在保护人类受试者免于登革热病中的功效。

[0067] 本发明涉及登革热病毒血清型 2 疫苗组合物，其包含：

(i) 选自以下的登革热抗原：

(a) 活的减毒登革热病毒；

(b) 灭活的登革热病毒；

(c) 活的减毒或灭活的嵌合登革热病毒；

(d) 登革热病毒样颗粒 (VLP)；和

(e) (a)-(d) 的两种或更多种的组合；

或

(ii) 能够在人细胞中表达登革热抗原的核酸构建体或病毒载体，所述登革热抗原是登革热 VLP ；

其中所述登革热抗原包含与 SEQ ID NO: 12 具有至少 90% 同一性的多肽。

[0068] 在优选的实施方案中，所述多肽与 SEQ ID NO: 12 具有至少 92%、至少 94%、至少 96%、至少 98%、至少 99%、至少 99.5% 同一性或 100% 同一性。

[0069] 优选所述登革热抗原选自活的减毒登革热病毒和活的减毒或灭活嵌合登革热病毒。优选所述登革热抗原选自活的减毒登革热病毒和活的减毒嵌合登革热病毒。优选所述登革热抗原是活的减毒嵌合登革热病毒。

[0070] 优选本发明的所述登革热抗原包含在 SEQ ID NO: 12 的全长内与 SEQ ID NO: 12 具有至少 90% 同一性的多肽，例如与 SEQ ID NO: 12 具有至少 92%、至少 94%、至少 96%、至少 98%、至少 99%、至少 99.5% 同一性或 100% 同一性。

[0071] 优选所述登革热抗原不含本文定义的 CYD-2 的 prM-E 序列。

[0072] 优选所述疫苗组合物不含 CYD-2。

[0073] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 251 位的多肽内的位置上包含缬氨酸残基的多肽。

[0074] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 6 位的多肽内的位置上包含甲硫氨酸残基的多肽。

[0075] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 129 位的多肽内的位置上包含缬氨酸残基的多肽。

[0076] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 129 位的多肽内的位置上包含异亮氨酸残基的多肽。

[0077] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 141 位的多肽内的位置上包含异亮氨酸残基的多肽。

[0078] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 164 位的多肽内的位置上包含异亮氨酸残基的多肽。

[0079] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 203 位的多肽内的位置上包含天冬氨酸残基的多肽。

[0080] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 203 位的多肽内的位置上包含天冬酰胺残基的多肽。

[0081] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 226 位的多肽内的位置上包含苏氨酸残基的多肽。

[0082] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 228 位的多肽内的位置上包含甘氨酸残基的多肽。

[0083] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 308 位的多肽内的位置上包含异亮氨酸残基的多肽。

[0084] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 308 位的多肽内的位置上包含缬氨酸残基的多肽。

[0085] 优选所述登革热抗原包含在相当于 SEQ ID NO: 12 的 478 位的多肽内的位置上包含苏氨酸残基的多肽。

[0086] 优选所述登革热抗原包含在相当于SEQ ID NO: 12的484位的多肽内的位置上包含缬氨酸残基的多肽。

[0087] 优选所述登革热抗原包含在相当于SEQ ID NO: 12的484位的多肽内的位置上包含异亮氨酸残基的多肽。

[0088] 优选所述登革热抗原包含在相当于SEQ ID NO: 12的485位的多肽内的位置上包含异亮氨酸残基的多肽。

[0089] 优选所述登革热抗原包含在相当于SEQ ID NO: 12的491位的多肽内的位置上包含丙氨酸残基的多肽。

[0090] 优选所述登革热抗原包含与SEQ ID NO: 3具有至少90%序列同一性的多肽。

[0091] 在优选的实施方案中，所述多肽与SEQ ID NO: 3具有至少92%、至少94%、至少96%、至少98%、至少99%、至少99.5%同一性或100%同一性。

[0092] 优选本发明的组合物包含与SEQ ID NO: 3具有至少90%、至少92%、至少94%、至少96%、至少98%、至少99%、至少99.5%同一性或100%同一性的多肽。优选本发明的登革热抗原包含在SEQ ID NO: 12的全长内与SEQ ID NO: 12具有至少90%同一性的多肽，例如与SEQ ID NO: 12具有至少92%、至少94%、至少96%、至少98%、至少99%、至少99.5%同一性或100%同一性。优选所述登革热抗原包含在相当于SEQ ID NO: 3的15位的多肽内的位置上包含甘氨酸残基的多肽。

[0093] 优选所述登革热抗原包含在相当于SEQ ID NO: 3的15位的多肽内的位置上包含丝氨酸残基的多肽。

[0094] 优选所述登革热抗原包含在相当于SEQ ID NO: 3的24位的多肽内的位置上包含亮氨酸残基的多肽。

[0095] 优选所述登革热抗原包含在相当于SEQ ID NO: 3的39位的多肽内的位置上包含异亮氨酸残基的多肽。

[0096] 优选所述登革热抗原包含在相当于SEQ ID NO: 3的39位的多肽内的位置上包含甲硫氨酸残基的多肽。

[0097] 优选所述登革热抗原包含在相当于SEQ ID NO: 3的120位的多肽内的位置上包含缬氨酸残基的多肽。

[0098] 优选所述登革热抗原包含在相当于SEQ ID NO: 3的120位的多肽内的位置上包含丙氨酸残基的多肽。

[0099] 优选所述登革热抗原包含在相当于SEQ ID NO: 3的125位的多肽内的位置上包含苏氨酸残基的多肽。

[0100] 优选本文定义的多肽（如本发明的疫苗组合物内所包含的登革热抗原内所包含的）包含在相当于SEQ ID NO: 3的125位的多肽内的位置上的苏氨酸残基和在相当于SEQ ID NO: 3的417位的多肽内的位置上的缬氨酸残基。

[0101] 优选由本文定义的核苷酸序列编码的多肽（即如本发明的疫苗组合物内所包含的）包含相当于SEQ ID NO: 3的24位的多肽内的位置上的亮氨酸残基、在相当于SEQ ID NO: 3的125位的多肽内的位置上的苏氨酸残基和在相当于SEQ ID NO: 3的417位的多肽内的位置上的缬氨酸残基。

[0102] 优选多肽（如本发明的疫苗组合物内所包含的登革热抗原内所包含的）包含(i)

SEQ ID NO: 13 所示序列或相对于如 SEQ ID NO: 13 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(ii) SEQ ID NO: 14 所示序列或相对于 SEQ ID NO: 14 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(iii) 如 SEQ ID NO: 15 所示序列或相对于 SEQ ID NO: 15 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(iv) SEQ ID NO: 16 所示序列或相对于 SEQ ID NO: 16 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(v) SEQ ID NO: 18 所示序列或相对于 SEQ ID NO: 18 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;或 (vi) SEQ ID NO: 26 所示序列或相对于 SEQ ID NO: 26 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列。优选当所述序列包含氨基酸取代时,所述序列具有至少 1 个且不多于 4 个氨基酸取代、优选至少 1 个且不多于 3 个氨基酸取代、优选 1 或 2 个氨基酸取代、优选 1 个氨基酸取代。优选至多 2 个、优选 1 个、优选无一取代是高影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 > 25 的评分) ;优选至多 3 个、优选 2 个、优选 1 个、优选无一取代是中等影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 >10-25 的评分) ;优选至多 5 个、优选 4 个、优选 3 个、优选 2 个、优选 1 个、优选无一取代是低影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 >0-10 的评分) ;优选所有所述取代是无影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 0 分)。优选所述取代不发生在相当于 SEQ ID NO: 12 的 226、228 和 251 位的所述序列内的位置上。优选当用于四价组合物的情况下时,包含所述多肽的登革热抗原导致平衡的免疫应答。优选当包含含有所述多肽的登革热抗原的疫苗组合物还包含本文定义的血清型 1、3 和 4 的登革热抗原时,所述疫苗组合物当给予哺乳动物 (优选人) 时产生平衡的免疫应答。

[0103] 优选所述登革热抗原包含含有选自以下序列的多肽 :SEQ ID NO: 13、SEQ ID NO: 14、SEQ ID NO: 15、SEQ ID NO: 16、SEQ ID NO: 18 和 SEQ ID NO: 26。

[0104] 优选所述登革热抗原包含含有选自以下序列的多肽 :SEQ ID NO: 13 和 SEQ ID NO: 16。

[0105] 优选登革热抗原 (如本发明的疫苗组合物所包含的) 包含含有以下的多肽 :(i) SEQ ID NO: 19 所示序列或相对于 SEQ ID NO: 19 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(ii) SEQ ID NO: 20 所示序列或相对于 SEQ ID NO: 20 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(iii) SEQ ID NO: 21 所示序列或相对于 SEQ ID NO: 21 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(iv) SEQ ID NO: 22 所示序列或相对于 SEQ ID NO: 22 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;(v) SEQ ID NO: 23 所示序列或相对于 SEQ ID NO: 23 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列 ;或 (vi) SEQ ID NO: 27 所示序列或相对于 SEQ ID NO: 27 所示序列具有至少 1 个且不多于 5 个氨基酸取代的序列。优选当所述序列包含氨基酸取代时,所述序列具有至少 1 个且不多于 4 个氨基酸取代、优选至少 1 个且不多于 3 个氨基酸取代、优选 1 或 2 个氨基酸取代、优选 1 个氨基酸取代。优选至多 2 个、优选 1 个、优选无一取代是高影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 > 25 的评分) ;优选至多 3 个、优选 2 个、优选 1 个、优选无一取代是中等影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 >10-25 的评分) ;优选至多 5 个、优选 4 个、优选 3 个、优选 2 个、优选 1 个、优选无一取代是低影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 >0-10 的评

分）;优选所有所述取代是无影响氨基酸取代（即在实施例 2 中公开的影响评分方法中达到 0 分）。优选所述取代不发生在相当于 SEQ ID NO: 3 的 125 位的所述序列内的位置处。优选当用于四价组合物的情况下时，包含所述多肽的登革热抗原导致平衡的免疫应答。优选当包含含有所述多肽的登革热抗原的疫苗组合物还包含本文定义的血清型 1、3 和 4 的登革热抗原时，所述疫苗组合物当给予哺乳动物（优选人）时产生平衡的免疫应答。

[0106] 优选登革热抗原（如本发明的疫苗组合物所包含的）包含含有选自以下序列的多肽 :SEQ ID NO: 19、SEQ ID NO: 20、SEQ ID NO: 21、SEQ ID NO: 22、SEQ ID NO: 23 和 SEQ ID NO: 27。

[0107] 优选登革热抗原（如本发明的疫苗组合物所包含的）包含 :

i) 具有 SEQ ID NO: 13 所示序列的多肽或相对于 SEQ ID NO: 13 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 19 所示序列的多肽或相对于 SEQ ID NO: 19 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;

ii) 具有 SEQ ID NO: 14 所示序列的多肽或相对于 SEQ ID NO: 14 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 20 所示序列的多肽或相对于 SEQ ID NO: 20 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;

iii) 具有 SEQ ID NO: 15 所示序列的多肽或相对于 SEQ ID NO: 15 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 21 所示序列的多肽或相对于 SEQ ID NO: 21 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;

iv) 具有 SEQ ID NO: 16 所示序列的多肽或相对于 SEQ ID NO: 16 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 22 所示序列的多肽或相对于 SEQ ID NO: 22 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;

v) 具有 SEQ ID NO: 18 所示序列的多肽或相对于 SEQ ID NO: 18 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 23 所示序列的多肽或相对于 SEQ ID NO: 23 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;或

vi) 具有 SEQ ID NO: 26 所示序列的多肽或相对于 SEQ ID NO: 26 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽 ;和

具有 SEQ ID NO: 27 所示序列的多肽或相对于 SEQ ID NO: 27 所示序列具有至少 1 个且不多于 5 个氨基酸取代的多肽。

[0108] 优选当所述序列包含氨基酸取代时，所述序列具有至少 1 个且不多于 4 个氨基酸取代、优选至少 1 个且不多于 3 个氨基酸取代、优选 1 或 2 个氨基酸取代、优选 1 个氨基酸取代。优选至多 2 个、优选 1 个、优选无一取代是高影响氨基酸取代（即在实施例 2 中公开的影响评分方法中达到 >25 的评分）;优选至多 3 个、优选 2 个、优选 1 个、优选无一取代是中等影响氨基酸取代（即在实施例 2 中公开的影响评分方法中达到 >10-25 的评分）;优选至多 5 个、优选 4 个、优选 3 个、优选 2 个、优选 1 个、优选无一取代是低影响氨基酸取代（即

在实施例 2 中公开的影响评分方法中达到 >0-10 的评分）；优选所有所述取代是无影响氨基酸取代（即在实施例 2 中公开的影响评分方法中达到 0 分）。优选所述取代不发生在相当于 SEQ ID NO: 12 的 226、228 和 251 位的所述序列内的位置上和相当于 SEQ ID NO: 3 的 125 位的位置上。优选当用于四价组合物的情况下时，包含所述多肽的登革热抗原导致平衡的免疫应答。优选当包含含有所述多肽的登革热抗原的疫苗组合物还包含本文定义的血清型 1、3 和 4 的登革热抗原时，所述疫苗组合物当给予哺乳动物（优选人）时产生平衡的免疫应答。

[0109] 优选登革热抗原（如本发明的疫苗组合物所包含的）包含：i) SEQ ID NO: 13 的多肽和 SEQ ID NO: 19 的多肽；ii) SEQ ID NO: 14 的多肽和 SEQ ID NO: 20 的多肽；iii) SEQ ID NO: 15 的多肽和 SEQ ID NO: 21 的多肽；iv) SEQ ID NO: 16 的多肽和 SEQ ID NO: 22 的多肽；v) SEQ ID NO: 18 的多肽和 SEQ ID NO: 23 的多肽或 vi) SEQ ID NO: 26 的多肽和多 SEQ ID NO: 27 的多肽。

[0110] 优选所述登革热抗原包含含有选自以下序列的多肽：SEQ ID NO: 8、SEQ ID NO: 9、SEQ ID NO: 10 和 SEQ ID NO: 11。

[0111] 优选所述登革热抗原包含含有选自以下序列的多肽：SEQ ID NO: 8 和 SEQ ID NO: 11。

[0112] 优选本发明的组合物包含选自以下的登革热抗原：(a) 活的减毒登革热病毒；(b) 灭活的登革热病毒；(c) 活的减毒或灭活的嵌合登革热病毒；或 (d) (a)-(c) 的两种或更多种的组合；其中所述登革热抗原包含编码包含本文定义的多肽的多肽的核苷酸序列。

[0113] 本发明还涉及包含选自以下血清型 2 的登革热抗原的疫苗组合物：(a) 活的减毒登革热病毒；(b) 灭活的登革热病毒；(c) 活的减毒或灭活的嵌合登革热病毒；或 (d) (a)-(c) 的两种或更多种的组合；其中所述登革热抗原包含与选自以下序列具有至少 90% 序列同一性的核苷酸序列：SEQ ID NO: 1 的 RNA 等同物、SEQ ID NO: 4 的 RNA 等同物、SEQ ID NO: 5 的 RNA 等同物、SEQ ID NO: 6 的 RNA 等同物、SEQ ID NO: 7 的 RNA 等同物和 SEQ ID NO: 25。提及与 SEQ ID NO: 1 的 RNA 等同物、SEQ ID NO: 4 的 RNA 等同物、SEQ ID NO: 5 的 RNA 等同物、SEQ ID NO: 6 的 RNA 等同物、SEQ ID NO: 7 的 RNA 等同物或 SEQ ID NO: 25 有至少 90% 序列同一性优选可在本文解读为至少 92%、至少 93%、至少 94%、至少 95%、至少 96%、至少 97%、至少 98%、至少 99%、至少 99.5% 或 100% 序列同一性。当本发明的该实施方案的核苷酸序列编码包含相对于由 SEQ ID NO: 1、SEQ ID NO: 4、SEQ ID NO: 5、SEQ ID NO：6、SEQ ID NO: 7 和 SEQ ID NO: 25 编码的多肽的一个或多个氨基酸取代的多肽时，优选至多 2 个、优选 1 个、优选无一取代是高影响氨基酸取代（即在实施例 2 中公开的影响评分方法中达到 >25 的评分）；优选至多 3 个、优选 2 个、优选 1 个、优选无一取代是中等影响氨基酸取代（即在实施例 2 中公开的影响评分方法中达到 >10-25 的评分）；优选至多 5 个、优选 4 个、优选 3 个、优选 2 个、优选 1 个、优选无一取代是低影响氨基酸取代（即在实施例 2 中公开的影响评分方法中达到 >0-10 的评分）；优选所有所述取代是无影响氨基酸取代（即在实施例 2 中公开的影响评分方法中达到 0 分）。优选所述取代不发生在相当于 SEQ ID NO: 12 的 226、228 和 251 位的所述多肽内的位置和相当于 SEQ ID NO: 3 的 24 和 125 位的所述多肽内的位置上。优选包含本发明的该实施方案的血清型 2 的登革热抗原的疫苗组合物当用于四价组合物的情况下时导致平衡的免疫应答。优选本发明的该实施方案的血

清型 2 的登革热抗原还包含本文其它部分描述的血清型 1 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原。优选当包含本发明的该实施方案的血清型 2 的登革热抗原的疫苗组合物还包含本文定义的血清型 1、3 和 4 的登革热抗原时，所述疫苗组合物当给予哺乳动物（优选人）时产生平衡的免疫应答。当本发明的疫苗组合物包含含有与 SEQ ID NO: 7 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的血清型 2 的登革热抗原时，所述疫苗组合物优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 7 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述登革热抗原不是 CYD-MD，或 (ii) 包含是 CYD-MD 的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 7 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述疫苗组合物不含 MD1280 的 prM-E 序列，或 (ii) 包含 MD1280 的 prM-E 序列的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 7 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-MD 的 M 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-MD 的 M 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 7 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 MD1280 的 prM-E 序列 (SEQ ID NO: 11) 产生的血清型 2 的嵌合登革热病毒，或 (ii) 包含使用 MD1280 的 prM-E 序列 (SEQ ID NO: 11) 产生的血清型 2 的嵌合登革热病毒的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 7 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-MD 的 prM 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-MD 的 prM 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 7 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 SEQ ID NO: 16 的多肽和 SEQ ID NO: 22 的多肽的登革热抗原（或包含编码包含所述多肽的蛋白质的核苷酸序列的登革热抗原），或 (ii) 包含含有 SEQ ID NO: 16 的多肽和 SEQ ID NO: 22 的多肽的登革热抗原（或包含编码包含所述多肽的蛋白质的核苷酸序列的登革热抗原）的疫苗组合物。优选包含含有与 SEQ ID NO: 7 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的血清型 2 的登革热抗原的本发明的疫苗组合物不含 :(i) 包含 MD1280 的 prM 和 E 氨基酸序列的嵌合病毒，或 (ii) 包含 CYD-MD 的 prM-E 序列 (SEQ ID NO: 11) 的血清型 2 的登革热抗原。当本发明的疫苗组合物包含含有与 SEQ ID NO: 4 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的血清型 2 的登革热抗原时，所述疫苗组合物优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 4 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述登革热抗原不是 CYD-LAV，或 (ii) 包含是 CYD-LAV 的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 4 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述疫苗组合物不含 LAV2 的 prM-E 序列，或 (ii) 包含 LAV2 的 prM-E 序列的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 4 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-LAV 的 M 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-LAV 的 M 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还

优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 4 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物,其中所述疫苗组合物不含使用 LAV2 的 prM-E 序列 (SEQ ID NO: 8) 产生的血清型 2 的嵌合登革热病毒,或 (ii) 包含使用 LAV2 的 prM-E 序列 (SEQ ID NO: 8) 产生的血清型 2 的嵌合登革热病毒的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 4 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物,其中所述疫苗组合物不含包含 CYD-LAV 的 prM 和 E 序列的登革热抗原,或 (ii) 包含含有 CYD-LAV 的 prM 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有与 SEQ ID NO: 4 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的登革热抗原的疫苗组合物,其中所述疫苗组合物不含包含 SEQ ID NO: 13 的多肽和 SEQ ID NO: 19 的多肽的登革热抗原 (或包含编码包含所述多肽的蛋白质的核苷酸序列的登革热抗原),或 (ii) 包含含有 SEQ ID NO: 13 的多肽和 SEQ ID NO: 19 的多肽的登革热抗原 (或包含编码包含所述多肽的蛋白质的核苷酸序列的登革热抗原) 的疫苗组合物。优选包含含有与 SEQ ID NO: 4 的 RNA 等同物具有至少 90% 同一性的核苷酸序列的血清型 2 的登革热抗原的本发明的疫苗组合物不含 :(i) 包含 LAV2 的 prM 和 E 氨基酸序列的嵌合病毒,或 (ii) 包含 CYD-LAV 的 prM-E 序列 (SEQ ID NO: 8) 的血清型 2 的登革热抗原。

[0114] 本发明还涉及包含选自以下血清型 2 的登革热抗原的疫苗组合物 :(a) 活的减毒登革热病毒 ;(b) 灭活的登革热病毒 ;(c) 活的减毒或灭活的嵌合登革热病毒 ;或 (d) (a)-(c) 的两种或更多种的组合 ;其中所述登革热抗原包含相对于选自以下的序列具有至少 1 个且不多于 20 个核苷酸取代的核苷酸序列 :SEQ ID NO: 1 的 RNA 等同物、SEQ ID NO: 4 的 RNA 等同物、SEQ ID NO: 5 的 RNA 等同物、SEQ ID NO: 6 的 RNA 等同物、SEQ ID NO: 7 的 RNA 等同物和 SEQ ID NO: 25。当本发明的该实施方案的核苷酸序列编码相对于由 SEQ ID NO: 1、SEQ ID NO: 4、SEQ ID NO: 5、SEQ ID NO :6、SEQ ID NO: 7 和 SEQ ID NO: 25 的编码的多肽包含一个或多个氨基酸取代的多肽时,优选至多 2 个、优选 1 个、优选无一取代是高影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 >25 的评分) ;优选至多 3 个、优选 2 个、优选 1 个、优选无一取代是中等影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 >10-25 的评分) ;优选至多 5 个、优选 4 个、优选 3 个、优选 2 个、优选 1 个、优选无一取代是低影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 >0-10 的评分) ;优选所有所述取代是无影响氨基酸取代 (即在实施例 2 中公开的影响评分方法中达到 0 分)。优选所述取代不发生在相当于 SEQ ID NO: 12 的 226、228 和 251 位的所述多肽内的位置和相当于 SEQ ID NO: 3 的 24 和 125 位的所述多肽内的位置上。优选包含本发明的该实施方案的血清型 2 的登革热抗原的疫苗组合物当用于四价组合物的情况下时导致平衡的免疫应答。优选本发明的该实施方案的血清型 2 的登革热抗原还包含本文其它部分描述的血清型 1 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原。优选当包含本发明的该实施方案的血清型 2 的登革热抗原的疫苗组合物还包含本文定义的血清型 1、3 和 4 的登革热抗原时,所述疫苗组合物当给予哺乳动物 (优选人) 时产生平衡的免疫应答。

[0115] 当本发明的疫苗组合物包含含有与 SEQ ID NO: 12 具有至少 90%、至少 92%、至少 94%、至少 96%、至少 98%、至少 99%、至少 99.5% 或 100% 同一性的多肽的本文定义的血清型 2

的登革热抗原时，其中所述多肽包含在相当于 SEQ ID NO: 12 的 485 位的多肽内的位置上的异亮氨酸残基或在相当于 SEQ ID NO: 12 的 491 位的多肽位内置上的丙氨酸残基，所述疫苗组合物优选 (i) 不是 CYD-MD ；(ii) 不含 MD1280 的 prM-E 序列 ；(iii) 不含使用 MD1280 的 prM-E 序列 (SEQ ID NO: 11) 产生的血清型 2 的嵌合登革热病毒 ；(iv) 不含包含 CYD-MD 的 prM 和 E 序列的登革热抗原 ；(v) 不含包含 SEQ ID NO: 16 的多肽和 SEQ ID NO: 22 的多肽的登革热抗原 (或包含编码包含所述多肽的蛋白质的核苷酸序列的登革热抗原) ；(vi) 不含包含 MD1280 的 prM 和 E 氨基酸序列的嵌合病毒 ；(vii) 不含包含 CYD-MD 的 prM-E 序列 (SEQ ID NO: 11) 的血清型 2 的登革热抗原，和 / 或 (viii) 不含包含 CYD-MD 的 M 和 E 序列的血清型 2 的登革热抗原。当本发明的疫苗组合物包含含有与 SEQ ID NO: 12 具有至少 90%、至少 92%、至少 94%、至少 96%、至少 98%、至少 99%、至少 99.5% 或 100% 同一性的多肽的本文定义的血清型 2 的登革热抗原时，其中所述多肽包含相当于 SEQ ID NO: 12 的 6 位的多肽内的位置上的甲硫氨酸残基或相当于 SEQ ID NO: 12 的 478 位的多肽内的位置上的苏氨酸残基，所述疫苗组合物优选 (i) 不是 CYD-LAV ；(ii) 不含 LAV2 的 prM-E 序列 ；(iii) 不含使用 LAV2 的 prM-E 序列 (SEQ ID NO: 8) 产生的血清型 2 的嵌合登革热病毒 ；(iv) 不含包含 CYD-LAV 的 prM 和 E 序列的登革热抗原 ；(v) 不含包含 SEQ ID NO: 13 的多肽和 SEQ ID NO: 19 的多肽的登革热抗原 (或包含编码包含所述多肽的蛋白质的核苷酸序列的登革热抗原) ；(vi) 不含包含 LAV2 的 prM 和 E 氨基酸序列的嵌合病毒 ；(vii) 不含包含 CYD-LAV 的 prM-E 序列 (SEQ ID NO: 8) 的血清型 2 的登革热抗原和 / 或 (viii) 不含包含 CYD-LAV 的 M 和 E 序列的血清型 2 的登革热抗原。

[0116] 优选所述登革热抗原包含含有不多于 1 个、不多于 2 个、不多于 3 个、不多于 4 个、不多于 5 个、不多于 6 个、不多于 7 个、不多于 8 个、不多于 9 个、不多于 10 个、不多于 11 个或不多于 12 个少量氨基酸残基的多肽，其中在 prM-E 或 E 序列上的规定位置上的少量氨基酸残基定义为以小于血清型 2 的登革热病毒 prM-E 或 E 序列的 15% 出现在该位置上的氨基酸。

[0117] 优选本发明的登革热病是病毒学上已证实的登革热病。

[0118] 优选本发明的人类受试者小于 18 岁或小于 12 岁。例如，本发明的人类受试者可为 0-17 岁、0-11 岁、4-17 岁、4-11 岁、4-6 岁、6-8 岁、8-10 岁、2-8 岁、2-11 岁、2-14 岁、9-16 岁、12-17 岁或 18-45 岁。更优选本发明的人类受试者为 4-11 岁，2-14 岁或 9-16 岁。本发明的人类受试者可为至少 9 月龄或小于 9 月龄。例如本发明的人类受试者可为 9 个月 -16 岁、9 个月 -14 岁、9 个月 -11 岁或 9 个月 -8 岁。本发明的人类受试者可为至少 9 月龄，对本文定义的疫苗组合物的任何组分无严重变态反应史，无先天性或获得性免疫缺陷，无有症状的 HIV 感染，且所述受试者应未怀孕或哺乳。

[0119] 向其给予本发明的疫苗组合物的人受试者优选为有感染风险的人，例如在存在登革热的区域 (即登革热病区) 旅行的人或所述区域的居民。优选本发明的人受试者驻留在登革热病区。本发明的登革热病区包括大多数热带和亚热带，例如由 WHO 确定为地方病国家的任何国家。例如，本发明的登革热病区可包含列入热带或亚热带的那些美洲国家或其部分。本发明的登革热病区因此可包括以下的任一个或多个 ：巴西、委内瑞拉、哥伦比亚、厄瓜多尔、秘鲁、玻利维亚、巴拉圭、巴拿马、哥斯达黎加、尼加拉瓜、洪都拉斯、萨尔瓦多、危地马拉、伯利兹、墨西哥、美国和加勒比海岛屿。在一个具体的实施方案中，本发明的登革热病

区可包括：巴西、哥伦比亚、洪都拉斯、墨西哥和波多黎各。本发明的登革热病区还可包括在热带和亚热带内的南亚和大洋洲国家。本发明的登革热病区因此可包括以下的任一个或多个：印度、缅甸 (Myanmar/Burma)、泰国、老挝、越南、柬埔寨、印度尼西亚、马来西亚、新加坡、菲律宾、台湾、巴布亚新几内亚和澳大利亚。在本发明的登革热病区，野生型登革热病毒的特定血清型、毒株或基因型可为主要的传播毒株。例如，血清型 2 的登革热病毒可表征为具有亚洲 I 或亚洲 / 美洲基因型。亚洲 / 美洲基因型毒株的特征在于下列残基的至少 1 个、至少 2 个、至少 3 个、至少 4 个、至少 5 个或所有 6 个：分别在 prM-16、E-83、E-203、E-226、E-228 和 E-346 位上的 Arg、Asn、Asp、Thr、Gly 和 His（其中 prM-16 表示 prM 蛋白的第 16 位，E-83 表示 E 蛋白的第 83 位等）。亚洲 I 基因型毒株的特征在于下列残基的至少 1 个、至少 2 个、至少 3 个、至少 4 个、至少 5 个或所有 6 个：分别在 prM-16、E-83、E-203、E-226、E-228 和 E-346 位上的 Ile、Lys、Asn、Arg、Glu 和 Tyr（参见 Hang 等，PLoS NTD，4(7)：e757 的表 1）。本发明优选的登革热病区是其中具有亚洲 / 美洲基因型的登革热病毒是主要的传播毒株的登革热病区，即在所述登革热病区中至少 50%、至少 60%、至少 70%、至少 80%、至少 90%、至少 95% 或 100% 登革热病病例由具有亚洲 / 美洲基因型的登革热病毒引起。本发明优选的登革热病区是其中血清型 1、3 或 4 的任一种或多种的登革热病毒是主要传播血清型的登革热病区，即至少 50%、至少 60%、至少 70%、至少 80%、至少 90%、至少 95% 或 100% 的登革热病病例由血清型 1、3 或 4 的登革热病毒引起。

[0120] 可将本发明的疫苗组合物给予黄病毒免疫受试者，例如登革热免疫受试者，或可将本发明的疫苗组合物给予黄病毒幼稚受试者。有利的是，将本发明的疫苗组合物给予黄病毒免疫受试者，例如登革热免疫受试者。

[0121] 优选本发明的组合物，例如用于本发明方法的组合物，降低 DHF 的可能性或严重程度。可通过比较接受本发明的疫苗组合物的受试者组的 DHF 的病例数与未接受本发明疫苗组合物的受试者的对照组的 DHF 病例数来测量 DHF 可能性的降低（即感染 DHF 的概率的降低）。通过计算在接受本发明的疫苗组合物的受试者组中显示 I、II、III 或 IV 级各自的 DHF 的受试者数并比较这些数与来自未接受本发明疫苗组合物的受试者的对照组的等效数，可确定 DHF 严重程度的降低。例如，当与发生在未接受本发明疫苗组合物的受试者的对照组中的 I 级 DHF、II 级 DHF、III 级 DHF 和 IV 级 DHF 的等效病例数相比时，用于本发明方法的组合物优选减少接受疫苗的那些受试者中的 I 级 DHF 的病例数、II 级 DHF 的病例数、III 级 DHF 的病例数和 / 或 IV 级 DHF 的病例数。

[0122] 优选本发明的组合物，例如用于本发明方法的组合物，降低有症状的病毒学上已证实的登革热病的发生率或可能性。有利的是，本发明的组合物，例如用于本发明方法的组合物，降低由血清型 1、3 或 4 的登革热病毒引起的有症状的病毒学上已证实的登革热病的发生率或可能性。有利的是，本发明的组合物，例如用于本发明方法的组合物，降低由血清型 1、2、3 或 4 的登革热病毒引起的有症状的病毒学上已证实的登革热病的发生率或可能性。优选本发明的组合物，例如用于本发明方法的组合物，降低由病毒学上已证实的登革热病所致的住院率，即降低住院的病毒学上已证实的登革热病的发生率。例如本发明的组合物，例如用于本发明方法的组合物，降低因由血清型 1、3 或 4 的登革热病毒引起的病毒学上已证实的登革热病所致的住院率，即降低由血清型 1、3 或 4 的登革热病毒引起的住院的病毒学上已证实的登革热病的发生率。

[0123] 优选本发明的组合物，例如用于本发明方法的组合物，降低登革热病的发生率或可能性。有利的是，本发明的组合物，例如用于本发明方法的组合物，降低由血清型 1、3 或 4 的登革热病毒引起的登革热病的发生率或可能性。有利的是，本发明的组合物，例如用于本发明方法的组合物，降低由血清型 1、2、3 或 4 的登革热病毒引起的登革热病的发生率或可能性。优选本发明的组合物，例如用于本发明方法的组合物，降低因登革热病所致的住院率，即降低住院登革热病的发生率。例如本发明的组合物，例如用于本发明方法的组合物，降低因由血清型 1、3 或 4 的登革热病毒引起的登革热病所致的住院率，即降低由血清型 1、3 或 4 的登革热病毒引起的住院登革热病的发生率。

[0124] 本发明的疫苗组合物可以多剂量给予。本发明的疫苗组合物的剂量可以初始接种方案给予，接着加强接种。例如，本发明的疫苗组合物可以 1 剂、2 剂、3 剂或 3 剂以上的剂量（例如 4 剂）给予。优选第 1 剂和第 3 剂相隔约 12 个月给予。例如，本发明的初始接种方案以 3 剂给予，其中所述接种方案的第 1 剂和第 3 剂相隔约 12 个月给予。有利的是，本发明的疫苗组合物以第 1 剂、第 2 剂和第 3 剂给予。在这类实施方案中，所述第 1 剂和所述第 3 剂可相隔约 12 个月给予。例如，本发明的疫苗组合物可以第 1 剂、第 2 剂和第 3 剂给予，其中所述第 2 剂在所述第 1 剂后约 6 个月给予，且其中所述第 3 剂在所述第 1 剂后约 12 个月给予。或者，3 个剂量可在零月时、在约 3-4 个月时（例如在约 3 个半月时）和在约 12 个月时给予（即其中组合物的第 2 剂在第 1 剂后约 3 个半月时给予，且其中组合物的第 3 剂在第 1 剂后约 12 个月时给予的方案）。

[0125] 本发明的疫苗组合物可以 2 剂给予。优选第 1 剂和第 2 剂在第 1 剂后约 6-12 个月给予。优选第 2 剂在第 1 剂后 8 个月给予。优选第 2 剂在第 1 剂后约 8 个半月至 9 个月时给予。

[0126] 本发明的疫苗组合物可以单剂量给予。

[0127] 本文定义的登革热病可能由登革热病毒的 2 种血清型的任一种引起。例如，登革热病优选由血清型 1 或血清型 3 的登革热病毒、血清型 1 或血清型 4 的登革热病毒、血清型 3 或血清型 4 的登革热病毒、血清型 1 或血清型 2 的登革热病毒、血清型 2 或血清型 3 的登革热病毒、血清型 2 或血清型 4 的登革热病毒引起。本文定义的登革热病，优选由登革热病毒的 3 种血清型的任一种引起。例如，登革热病优选由血清型 1、2 或 3 的登革热病毒、血清型 1、3 或 4 的登革热病毒、血清型 1、2 或 4 的登革热病毒、血清型 2、3 或 4 的登革热病毒引起。在另一个实施方案中，登革热病由血清型 1 的登革热病毒、血清型 2 的登革热病毒、血清型 3 的登革热病毒或血清型 4 的登革热病毒引起。

[0128] 例如用于本发明方法的本发明的疫苗组合物优选包含血清型 1 的登革热抗原、血清型 2 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原。所述组合物在本文可描述为四价组合物。例如本发明的组合物，例如用于本发明的保护方法的组合物，可有利地包含血清型 1、2、3 和 4 的登革热抗原的下列组合的任一种 ：i) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-LAV 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的嵌合登革热病毒和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ；ii) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-BID 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ；(iii) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-PR 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列

的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ;(iv) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-MD 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原。例如,本发明的组合物还可有利地包含登革热抗原的下列组合的任一种 :i) CYD-1、CYD-LAV、CYD-3 和 CYD-4 ;ii) CYD-1、CYD-BID、CYD-3 和 CYD-4 ;(iii) CYD-1、CYD-PR、CYD-3 和 CYD-4 或 (iv) CYD-1、CYD-MD、CYD-3 和 CYD-4。本发明的组合物还可有利地包含登革热抗原的下列组合 :i) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、VDV2、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原。例如,本发明的组合物可有利地包含 CYD-1、VDV-2、CYD-3 和 CYD-4。如本文所述,本发明的组合物可有利地包含血清型 2 的登革热抗原,其包含 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列。如本文所述,本发明的组合物可有利地包含血清型 2 的登革热抗原,其包含与 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列具有至少 90% 同一性的序列。例如,所述序列可与 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列有至少 91%、至少 92%、至少 93%、至少 94%、至少 95%、至少 96%、至少 97%、至少 98% 或至少 99% 同一性。

[0129] 例如用于本发明方法的本发明的疫苗组合物优选包含血清型 1 的登革热抗原、血清型 2 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原。所述组合物在本文可描述为四价组合物。例如本发明的组合物,例如用于本发明的保护方法的组合物,可有利地包含血清型 1、2、3 和 4 的登革热抗原的下列组合的任一种 :i) 包含 CYD-1 的 M 和 E 序列的登革热抗原、包含 CYD-LAV 的 M 和 E 序列的登革热抗原、包含 CYD-3 的 M 和 E 序列的嵌合登革热病毒和包含 CYD-4 的 M 和 E 序列的登革热抗原 ;ii) 包含 CYD-1 的 M 和 E 序列的登革热抗原、包含 CYD-BID 的 M 和 E 序列的登革热抗原、包含 CYD-3 的 M 和 E 序列的登革热抗原和包含 CYD-4 的 M 和 E 序列的登革热抗原 ;(iii) 包含 CYD-1 的 M 和 E 序列的登革热抗原、包含 CYD-PR 的 M 和 E 序列的登革热抗原、包含 CYD-3 的 M 和 E 序列的登革热抗原和包含 CYD-4 的 M 和 E 序列的登革热抗原 ;(iv) 包含 CYD-1 的 M 和 E 序列的登革热抗原、包含 CYD-MD 的 M 和 E 序列的登革热抗原、包含 CYD-3 的 M 和 E 序列的登革热抗原和包含 CYD-4 的 M 和 E 序列的登革热抗原。例如,本发明的组合物还可有利地包含登革热抗原的下列组合的任一种 :i) CYD-1、CYD-LAV、CYD-3 和 CYD-4 ;ii) CYD-1、CYD-BID、CYD-3 和 CYD-4 ;(iii) CYD-1、CYD-PR、CYD-3 和 CYD-4 或 (iv) CYD-1、CYD-MD、CYD-3 和 CYD-4。本发明的组合物还可有利地包含登革热抗原的下列组合 :i) 包含 CYD-1 的 M 和 E 序列的登革热抗原、VDV2、包含 CYD-3 的 M 和 E 序列的登革热抗原和包含 CYD-4 的 M 和 E 序列的登革热抗原。例如,本发明的组合物可有利地包含 CYD-1、VDV-2、CYD-3 和 CYD-4。如本文所述,本发明的组合物可有利地包含血清型 2 的登革热抗原,其包含 CYD-LAV (SEQ ID NO: 13)、CYD-BID (SEQ ID NO: 14)、CYD-PR (SEQ ID NO: 15)、CYD-MD (SEQ ID NO: 16) 或 SEQ ID NO: 18 的 E 序列。在某些实施方案中,包含含有 SEQ ID NO: 18 所示序列的登革热抗原的本发明的组合物不是包含来自 SEQ ID NO: 2 的 prM-E 序列的血清型 2 的疫苗组合物。在某些实施方案中,本发明的组合物可为包含含有 SEQ ID NO: 18 所示序列的登革热抗原的组合物,其中

所述组合物不是包含使用 SEQ ID NO: 2 的 prM-E 序列产生的血清型 2 的嵌合登革热病毒的本发明的疫苗组合物，或它是包含使用 SEQ ID NO: 2 的 prM-E 序列产生的血清型 2 的嵌合登革热病毒的组合物。如本文所述，本发明的组合物可有利地包含血清型 2 的登革热抗原，其包含与 CYD-LAV (SEQ ID NO: 13)、CYD-BID (SEQ ID NO: 14)、CYD-PR (SEQ ID NO: 15)、CYD-MD (SEQ ID NO: 16) 或 SEQ ID NO: 18 的 E 序列具有至少 90% 同一性的序列。例如，所述序列可与 CYD-LAV (SEQ ID NO: 13)、CYD-BID (SEQ ID NO: 14)、CYD-PR (SEQ ID NO: 15)、CYD-MD (SEQ ID NO: 16) 或 SEQ ID NO: 18 的 E 序列有至少 91%、至少 92%、至少 93%、至少 94%、至少 95%、至少 96%、至少 97%、至少 98% 或至少 99% 同一性。

[0130] 如本文所述，本发明的组合物 (例如用于本发明方法的四价制剂)，可有利地包含血清型 2 的登革热抗原，其包含选自 SEQ ID NO: 19、SEQ ID NO: 20、SEQ ID NO: 21、SEQ ID NO: 22 或 SEQ ID NO: 23 的多肽。当本发明的疫苗组合物包含含有具有 SEQ ID NO: 19 的序列的多肽的血清型 2 的登革热抗原时，所述疫苗组合物优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 19 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含 CYD-LAV，或 (ii) 包含 CYD-LAV 的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 19 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-LAV 的 M 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-LAV 的 M 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 19 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-LAV 的 prM 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-LAV 的 prM 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 19 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 LAV-2 的 prM-E 序列产生的血清型 2 的嵌合登革热病毒，或 (ii) 包含使用 LAV-2 的 prM-E 序列 (SEQ ID NO: 8) 产生的血清型 2 的嵌合登革热病毒的疫苗组合物。优选其包含具有 SEQ ID NO: 19 的序列的多肽的本发明的疫苗组合物不含 :(i) 包含 LAV-2 的 prM 和 E 氨基酸序列的嵌合病毒 ;(ii) 包含 CYD-LAV 的 prM-E 序列 (SEQ ID NO: 8) 的血清型 2 的登革热抗原，或 (iii) 包含 LAV-2 的 prM-E 序列的登革热抗原。当本发明的疫苗组合物包含含有具有 SEQ ID NO: 21 的序列的多肽的血清型 2 的登革热抗原时，所述疫苗组合物优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 21 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含 CYD-PR，或 (ii) 包含 CYD-PR 的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 21 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-PR 的 prM 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-PR 的 prM 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 21 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 PR/DBO23 的 prM-E 序列 (SEQ ID NO: 10) 产生的血清型 2 的嵌合登革热病毒，或 (ii) 包含使用 PR/DBO23 的 prM-E 序列 (SEQ ID NO: 10) 产生的血清型 2 的嵌合登革热病毒的疫苗组合物。优选包含具有 SEQ ID NO: 21 的序列的多肽的本发明的疫苗组合物不含 :(i) 包含 PR/DBO23 的 prM 和 E 氨基酸序列的嵌合病毒 ;(ii) 包含 CYD-PR 的 prM-E 序列 (SEQ ID NO: 10) 的血清型 2 的登革热抗原，或 (iii) 包含 PR/DBO23 的 prM-E 序列的登革热抗原。当本发明的疫苗组合物包含含有具有 SEQ ID NO: 22 的序列的多肽的血清型 2 的登

革热抗原时，所述疫苗组合物优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 22 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含 CYD-MD，或 (ii) 包含 CYD-MD 的疫苗组合物。所述疫苗组合物还优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 22 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-MD 的 prM 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-MD 的 prM 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 22 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-MD 的 M 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-MD 的 M 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 22 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 MD1280 的 prM-E 序列 (SEQ ID NO: 11) 产生的血清型 2 的嵌合登革热病毒，或 (ii) 包含使用 MD1280 的 prM-E 序列 (SEQ ID NO: 11) 产生的血清型 2 的嵌合登革热病毒的疫苗组合物。优选包含具有 SEQ ID NO: 22 的序列的多肽的本发明的疫苗组合物不含：(i) 包含 MD1280 的 prM 和 E 氨基酸序列的嵌合病毒；(ii) 包含 CYD-MD 的 prM-E 序列 (SEQ ID NO: 11) 的血清型 2 的登革热抗原，或 (iii) 包含 MD1280 的 prM-E 序列的登革热抗原。当本发明的疫苗组合物包含含有具有 SEQ ID NO: 23 的序列的多肽的血清型 2 的登革热抗原时，所述疫苗组合物优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 23 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 SEQ ID NO: 2 的 prM-E 序列产生的血清型 2 的嵌合登革热病毒，或 (ii) 包含使用 SEQ ID NO: 2 的 prM-E 序列产生的血清型 2 的嵌合登革热病毒的疫苗组合物。优选含有具有 SEQ ID NO: 23 的序列的多肽的本发明的疫苗组合物不含：(i) 包含 SEQ ID NO: 2 的血清型 2 的登革热抗原，或 (ii) 包含来自 SEQ ID NO: 2 的 prM-E 序列的登革热抗原。

[0131] 优选本发明的血清型2的登革热抗原还包含选自SEQ ID NO: 13、SEQ ID NO: 14、SEQ ID NO: 15，SEQ ID NO: 16 或 SEQ ID NO: 18 的多肽。例如，所述血清型 2 的登革热抗原优选包含：i) SEQ ID NO: 13 的多肽和 SEQ ID NO: 19 的多肽；ii) SEQ ID NO: 14 的多肽和 SEQ ID NO: 20 的多肽；iii) SEQ ID NO: 15 的多肽和 SEQ ID NO: 21 的多肽；iv) SEQ ID NO: 16 的多肽和 SEQ ID NO: 22 的多肽；或 v) SEQ ID NO: 18 的多肽和 SEQ ID NO: 23 的多肽。当本发明的疫苗组合物包含含有具有 SEQ ID NO: 13 的序列的多肽和具有 SEQ ID NO: 19 的序列的多肽的血清型2的登革热抗原时，所述疫苗组合物优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 13 的序列的多肽和具有 SEQ ID NO: 19 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含 CYD-LAV，或 (ii) 包含 CYD-LAV 的疫苗组合物。所述疫苗组合物还优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 13 的序列的多肽和具有 SEQ ID NO: 19 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-LAV 的 prM 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-LAV 的 prM 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 13 的序列的多肽和具有 SEQ ID NO: 19 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-LAV 的 M 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-LAV 的 M 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个：(i) 包含含有具有 SEQ ID NO: 13 的序列的多肽和具有 SEQ ID NO: 19 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 LAV-2 的 prM-E 序列产生的血

清型 2 的嵌合登革热病毒，或 (ii) 包含使用 LAV-2 的 prM-E 序列 (SEQ ID NO: 8) 产生的血清型 2 的嵌合登革热病毒的疫苗组合物。优选包含具有 SEQ ID NO: 13 的序列的多肽和具有 SEQ ID NO: 19 的序列的多肽的本发明的疫苗组合物不含 :(i) 包含 LAV-2 的 prM 和 E 氨基酸序列的嵌合病毒 ;(ii) 包含 CYD-LAV 的 prM-E 序列 (SEQ ID NO: 8) 的血清型 2 的登革热抗原，或 (iii) 包含 LAV-2 的 prM-E 序列的登革热抗原。当本发明的疫苗组合物包含含有具有 SEQ ID NO: 15 的序列的多肽和具有 SEQ ID NO: 21 的序列的多肽的血清型 2 的登革热抗原时，所述疫苗组合物优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 15 的序列的多肽和具有 SEQ ID NO: 21 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含 CYD-PR，或 (ii) 包含 CYD-PR 的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含具有 SEQ ID NO: 15 的序列的多肽和包含具有 SEQ ID NO: 21 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-PR 的 prM 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-PR 的 prM 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 15 的序列的多肽和具有 SEQ ID NO: 21 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 PR/DB023 的 prM-E 序列 (SEQ ID NO: 10) 产生的血清型 2 的嵌合登革热病毒，或 (ii) 包含使用 PR/DB023 的 prM-E 序列 (SEQ ID NO: 10) 产生的血清型 2 的嵌合登革热病毒的疫苗组合物。优选包含具有 SEQ ID NO: 15 的序列的多肽和具有 SEQ ID NO: 21 的序列的多肽的本发明的疫苗组合物不含 :(i) 包含 PR/DB023 的 prM 和 E 氨基酸序列的嵌合病毒 ;(ii) 包含 CYD-PR 的 prM-E 序列 (SEQ ID NO: 10) 的血清型 2 的登革热抗原或 (iii) 包含 PR/DB023 的 prM-E 序列的登革热抗原。当本发明的疫苗组合物包含含有具有 SEQ ID NO: 16 的序列的多肽和具有 SEQ ID NO: 22 的序列的多肽的血清型 2 的登革热抗原时，所述疫苗组合物优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 16 的序列的多肽和具有 SEQ ID NO: 22 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含 CYD-MD，或 (ii) 包含 CYD-MD 的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含具有 SEQ ID NO: 16 的序列的多肽和包含具有 SEQ ID NO: 22 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-MD 的 prM 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-MD 的 prM 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 16 的序列的多肽和具有 SEQ ID NO: 22 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含包含 CYD-MD 的 M 和 E 序列的登革热抗原，或 (ii) 包含含有 CYD-MD 的 M 和 E 序列的登革热抗原的疫苗组合物。所述疫苗组合物还优选为以下任一个 :(i) 包含含有具有 SEQ ID NO: 16 的序列的多肽和具有 SEQ ID NO: 22 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 MD1280 的 prM-E 序列 (SEQ ID NO: 11) 产生的血清型 2 的嵌合登革热病毒，或 (ii) 包含使用 MD1280 的 prM-E 序列 (SEQ ID NO: 11) 产生的血清型 2 的嵌合登革热病毒的疫苗组合物。优选包含具有 SEQ ID NO: 16 的序列的多肽和具有 SEQ ID NO: 22 的序列的多肽的本发明的疫苗组合物不含 :(i) 包含 MD1280 的 prM 和 E 氨基酸序列的嵌合病毒 ;(ii) 包含 CYD-MD 的 prM-E 序列 (SEQ ID NO: 11) 的血清型 2 的登革热抗原，或 (iii) 包含 MD1280 的 prM-E 序列的登革热抗原。当本发明的疫苗组合物包含含有具有 SEQ ID NO: 18 的序列的多肽和具有 SEQ ID NO: 23 的序列的多肽的血清型 2 的登革热抗原时，所述疫苗组合物优选为以下任一个 :(i) 包含具

有 SEQ ID NO: 18 的序列的多肽和具有 SEQ ID NO: 23 的序列的多肽的登革热抗原的疫苗组合物，其中所述疫苗组合物不含使用 SEQ ID NO: 2 的 prM-E 序列产生的血清型 2 的嵌合登革热病毒，或 (ii) 包含使用 SEQ ID NO: 2 的 prM-E 序列产生的血清型 2 的嵌合登革热病毒的疫苗组合物。优选包含具有 SEQ ID NO: 18 的序列的多肽和具有 SEQ ID NO: 23 的序列的多肽的本发明的疫苗组合物不含 :(i) 包含 SEQ ID NO: 2 的血清型 2 的登革热抗原，或 (ii) 包含来自 SEQ ID NO: 2 的 prM-E 序列的登革热抗原。

[0132] 本文所述的本发明的组合物 (例如用于本发明方法的四价制剂)，可有利地包含血清型 2 的登革热抗原，其包含与 SEQ ID NO: 19、SEQ ID NO: 20、SEQ ID NO: 21、SEQ ID NO: 22 或 SEQ ID NO: 23 具有至少 90% 同一性的多肽。优选所述血清型 2 的登革热抗原还包含与 SEQ ID NO: 13、SEQ ID NO: 14、SEQ ID NO: 15、SEQ ID NO: 16 或 SEQ ID NO: 18 具有至少 90% 同一性的多肽。例如，所述血清型 2 的登革热抗原优选包含 :i) 与 SEQ ID NO: 13 具有至少 90% 序列同一性的多肽和与 SEQ ID NO: 19 具有至少 90% 序列同一性的多肽 ;ii) 与 SEQ ID NO: 14 具有至少 90% 序列同一性的多肽和与 SEQ ID NO: 20 具有至少 90% 序列同一性的多肽 ;iii) 与 SEQ ID NO: 15 具有至少 90% 序列同一性的多肽和与 SEQ ID NO: 21 具有至少 90% 序列同一性的多肽 ;iv) 与 SEQ ID NO: 16 具有至少 90% 序列同一性的多肽和与 SEQ ID NO: 22 具有至少 90% 序列同一性的多肽 ;或 v) 与 SEQ ID NO: 18 具有至少 90% 序列同一性的多肽和与 SEQ ID NO: 23 具有至少 90% 序列同一性的多肽。在优选的实施方案中，本文提及至少 90% 同一性时可解释为与指定序列有至少 91%、至少 92%、至少 93%、至少 94%、至少 95%、至少 96%、至少 97%、至少 98% 或至少 99% 同一性。

[0133] 在前面段落描述的血清型 2 的登革热抗原可有利地与本文其它部分描述的血清型 1、3 和 4 的登革热抗原的任一种组合形成包含血清型 1 的登革热抗原、血清型 2 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原的四价制剂。例如，血清型 1、3 和 4 的登革热抗原可各自独立地自活的减毒登革热病毒、灭活的登革热病毒、活的减毒或灭活的嵌合登革热病毒或登革热病毒样颗粒 (VLP)。优选所述血清型 1、3 和 4 的登革热抗原各自独立选自活的减毒登革热病毒和活的减毒嵌合登革热病毒。优选所述血清型 1、3 和 4 的登革热抗原分别是血清型 1、3 和 4 的活的减毒嵌合登革热病毒。优选所述血清型 1、3 和 4 的活的减毒嵌合登革热病毒各自包含来自登革热病毒的一种或多种蛋白质和来自不同黄病毒的一种或多种蛋白质。例如，所述血清型 1、3 和 4 的活的减毒嵌合登革热病毒各自有利地为 YF/ 登革热嵌合体。优选所述血清型 1、3 和 4 的登革热抗原各自为活的减毒嵌合登革热病毒，其中受体黄病毒的遗传骨架通过编码受体黄病毒 prM 和 E 蛋白的序列用登革热病毒的相应序列交换而被修饰。优选所述受体黄病毒是黄热病病毒。例如，在一个有利的实施方案中，所述血清型 1、3 和 4 的活的减毒嵌合登革热病毒分别是 Chimerivax 登革热血清型 1 毒株 (即 CYD-1 毒株)、Chimerivax 登革热血清型 3 毒株 (即 CYD-3 毒株) 和 Chimerivax 登革热血清型 4 毒株 (即 CYD-4 毒株)。

[0134] 本发明人的目的是提供优化的四价登革热疫苗组合物 (即包含血清型 1、2、3 和 4 各自的登革热抗原的疫苗组合物)，当与由实施例 1 限定的 CYD-1、CYD-2、CYD-3 和 CYD-4 产生的中和抗体反应相比时，其提供针对血清型 2 的登革热病毒的改进的中和抗体反应。

[0135] 因此，一方面，本发明有利地提供疫苗组合物，其中所述组合物包含血清型 1、2、3 和 4 各自的登革热抗原，其中所述血清型 1、3 和 4 的登革热抗原各自为活的减毒嵌合登革

热病毒，且所述血清型 2 的登革热抗原为活的减毒登革热病毒，其包含与 SEQ ID NO: 24 所示序列具有至少 90% 序列同一性的核酸序列。

[0136] 因此，另一方面，本发明有利地提供疫苗组合物，其包含血清型 1 的登革热抗原、血清型 2 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原，其中：

i) 所述血清型 1 的登革热抗原是 YF/ 登革热嵌合登革热病毒（即其中 YF 病毒的遗传骨架通过编码 YF 病毒的 prM 和 E 蛋白的序列用登革热血清型 1 病毒的相应序列交换而被修饰的受体黄热病病毒）；

ii) 所述血清型 2 的登革热抗原是活的减毒血清型 2 的登革热病毒，其包含与 SEQ ID NO: 24 所示序列具有至少 90% 序列同一性的核酸序列；

iii) 所述血清型 3 的登革热抗原是 YF/ 登革热嵌合登革热病毒（即其中 YF 病毒的遗传骨架通过编码 YF 病毒的 prM 和 E 蛋白的序列用登革热血清型 3 病毒的相应序列交换而被修饰的受体黄热病病毒）和

iv) 所述血清型 4 的登革热抗原是 YF/ 登革热嵌合登革热病毒（即其中 YF 病毒的遗传骨架通过编码 YF 病毒的 prM 和 E 蛋白的序列用登革热血清型 4 病毒的相应序列交换而被修饰的受体黄热病病毒）。

[0137] 优选所述受体 YF 病毒（其形成血清型 1、3 和 4 的 YF/ 登革热嵌合病毒的遗传骨架）是减毒 YF 病毒。例如，所述受体 YF 病毒可以是选自 YF 17D、YF 17DD 和 YF 17D204 的减毒 YF 病毒。优选血清型 1、3 和 4 的 YF/ 登革热嵌合病毒分别为 Chimerivax 登革热血清型 1（即 CYD-1）、Chimerivax 登革热血清型 3（即 CYD-3）和 Chimerivax 登革热血清型 4（即 CYD-4）。

[0138] 本文提及与 SEQ ID NO: 24 所示序列具有至少 90% 序列同一性的核酸序列可优选解读为与 SEQ ID NO: 24 所示序列具有至少 92%、至少 94%、至少 96%、至少 98%、至少 99% 或 100% 序列同一性的核酸序列。优选相当于 SEQ ID NO: 24 的 736、1619、4723、5062、9191、10063、10507、57、524、2055、2579、4018、5547、6599 和 8571 位的所述核酸序列（与 SEQ ID NO: 24 所示序列具有至少 90% 序列同一性）内的位置上的核苷酸不突变。有利的是，是用于本发明的组合物中的活的减毒登革热病毒的血清型 2 的登革热抗原（例如与上文和本文其它部分描述的血清型 1、3 和 4 的登革热抗原（例如是活的减毒嵌合登革热病毒的血清型 1、3 和 4 的登革热抗原，例如 YF/ 登革热嵌合登革热病毒）联用）是包含与 SEQ ID NO: 24 所示序列具有 100% 序列同一性的核酸序列的活的减毒登革热病毒或当与 SEQ ID NO: 24 所示序列比较时包含至少 1 个且不多于 20 个核苷酸取代的活的减毒登革热病毒。优选当与 SEQ ID NO: 24 所示序列比较时，所述活的减毒登革热病毒包含至少一个且不多于 15、14、13、12、11、10、9、8、7、6、5、4、3 或 2 个核苷酸取代。优选在相当于 SEQ ID NO: 24 的 736、1619、4723、5062、9191、10063、10507、57、524、2055、2579、4018、5547、6599 和 8571 位的所述核酸序列内的位置上的核苷酸不突变。有利的是，当与 SEQ ID NO: 24 所示序列比较时，是用于本发明的组合物中的活的减毒登革热病毒的血清型 2 的登革热抗原包含不多于 20 个碱基突变、缺失或插入的核酸序列。在某些情况下，当与 SEQ ID NO: 24 所示序列比较时，所述血清型 2 的活的减毒登革热病毒包含不多于 15 个或甚至不多于 12、11、10、9、8、7、6、5、4、3、2 或 1 个碱基突变、缺失或插入的核酸序列。优选在相当于 SEQ ID NO: 24 的 736、1619、4723、5062、9191、10063、10507、57、524、2055、2579、4018、5547、6599 和 8571 位上的

所述核酸序列内的位置上的核苷酸不突变。

[0139] 另外优选的是，用于本发明的疫苗组合物中的血清型 2 的登革热抗原（例如是血清型 2 的活的减毒登革热病毒或活的减毒嵌合登革热病毒的登革热抗原）当在四价登革热疫苗组合物的情况下使用时，能够诱导人的中和抗体并且能够诱导平衡的免疫应答。另外优选的是，用于本发明的疫苗组合物中的血清型 2 的登革热抗原（例如是血清型 2 的活的减毒登革热病毒或活的减毒嵌合登革热病毒的登革热抗原）在人中产生低病毒血症或无病毒血症。另外优选的是，当与由实施例 1 限定的 CYD-1、CYD-2、CYD-3 和 CYD-4 产生的中和抗体反应相比时，用于本发明的四价疫苗组合物的血清型 2 的登革热抗原（例如是血清型 2 的活的减毒登革热病毒或活的减毒嵌合登革热病毒的登革热抗原）提供针对血清型 2 的登革热病毒的改进的中和抗体反应。

[0140] 有利的是，用于本发明的组合物包含血清型 1、2、3 和 4 各自的登革热抗原，其中：(i) 所述血清型 1 的登革热抗原是 CYD-1 以外的活的减毒嵌合登革热病毒或所述血清型 1 的登革热抗原是 CYD-1；(ii) 所述血清型 2 的登革热抗原是 VDV-2 以外的活的减毒登革热病毒或所述血清型 2 的登革热抗原是 VDV-2；(iii) 所述血清型 3 的登革热抗原是 CYD-3 以外的活的减毒嵌合登革热病毒或所述血清型 3 的登革热抗原是 CYD-3，和 (iv) 所述血清型 4 的登革热抗原是 CYD-4 以外的活的减毒嵌合登革热病毒或所述血清型 4 的登革热抗原是 CYD-4。在这种情况下，VDV-2 毒株是通过对 Vero 细胞的后续适应而来源于 DEN-2 16681/PDK53 毒株 (LAV2) 的毒株，其中与包括 4 个沉默突变的 DEN-2 16681/PDK53 毒株相比，所述 VDV-2 毒株具有 10 个另外的突变。

[0141] 有利的是，用于本发明的组合物包含血清型 1、2、3 和 4 各自的登革热抗原，其中所述血清型 1、3 和 4 的登革热抗原各自为活的减毒嵌合登革热病毒，且所述血清型 2 的登革热抗原是包含与 SEQ ID NO: 24 所示序列具有至少 90% 序列同一性的核酸序列的活的减毒登革热病毒，且其中所述血清型 1、2、3 和 4 的登革热抗原分别不是 CYD-1、VDV-2、CYD-3 和 CYD-4 或分别不是包含 CYD-1 的 M 和 E 序列的登革热抗原、VDV2、包含 CYD-3 的 M 和 E 序列的登革热抗原和包含 CYD-4 的 M 和 E 序列的登革热抗原。

[0142] 有利的是，用于本发明的组合物包含血清型 1、2、3 和 4 各自的登革热抗原，其中：(i) 所述血清型 1 的登革热抗原是 CYD-1 以外的活的减毒嵌合登革热病毒或所述血清型 1 的登革热抗原是 CYD-1；(ii) 所述血清型 2 的登革热抗原是 VDV-2 以外的活的减毒登革热病毒或所述血清型 2 的登革热抗原是 VDV-2；(iii) 所述血清型 3 的登革热抗原是 CYD-3 以外的活的减毒嵌合登革热病毒或所述血清型 3 的登革热抗原是 CYD-3 和 (iv) 所述血清型 4 的登革热抗原是 CYD-4 以外的活的减毒嵌合登革热病毒或所述血清型 4 的登革热抗原是 CYD-4。在这种情况下，VDV-2 毒株是包含 SEQ ID NO: 24 所示核酸序列的毒株。

[0143] 本发明优选的疫苗组合物，例如用于本发明的方法，包含血清型 1 的登革热抗原、血清型 2 的登革热抗原、血清型 3 的登革热抗原和血清型 4 的登革热抗原，其中：

i) 所述血清型 1 的登革热抗原是 CYD-1 以外的 YF/ 登革热嵌合登革热病毒或所述血清型 1 的登革热抗原是 CYD-1；

ii) 所述血清型 2 的登革热抗原是包含与 SEQ ID NO: 24 所示序列具有至少 90% 序列同一性的核酸序列的活的减毒血清型 2 的登革热病毒，其中所述血清型 2 的登革热抗原不是包含与 SEQ ID NO: 24 所示序列具有 100% 序列同一性的核酸序列的活的减毒血清型 2 的

登革热病毒或所述血清型 2 的登革热抗原是包含与 SEQ ID NO: 24 所示序列具有 100% 序列同一性的核酸序列的活的减毒血清型 2 的登革热病毒 ;

iii) 所述血清型 3 的登革热抗原是 CYD-3 以外的 YF/ 登革热嵌合登革热病毒或所述血清型 3 的登革热抗原是 CYD-3 ;和

iv) 所述血清型 4 的登革热抗原是 CYD-4 以外的 YF/ 登革热嵌合登革热病毒或所述血清型 4 的登革热抗原是 CYD-4。

[0144] 有利的是,是用于本发明的疫苗组合物中的活减毒嵌合登革热病毒的血清型 2 的登革热抗原(例如与上文和本文其它部分描述的血清型 1、3 和 4 的登革热抗原(例如是 YF/ 登革热嵌合登革热病毒的血清型 1、3 和 4 的登革热抗原)联用)包含与 SEQ ID NO: 25 所示序列具有至少 90% 同一性的核酸序列。优选所述核酸序列与 SEQ ID NO: 25 所示序列具有至少 92%、至少 94%、至少 96%、至少 98%、至少 99% 或 100% 序列同一性。优选在相当于 SEQ ID NO: 24 的 524、736、1619 和 2055 位的所述核酸序列内的位置上的核苷酸不突变(即保留出现在这些位置上的 SEQ ID NO: 24 中的核苷酸)。

[0145] 有利的是,是用于本发明的疫苗组合物中的嵌合登革热病毒的血清型 2 的登革热抗原(例如与上文和本文其它部分描述的血清型 1、3 和 4 的登革热抗原(例如是 YF/ 登革热嵌合登革热病毒的血清型 1、3 和 4 的登革热抗原)联用)包含与 LAV-2 毒株的 prM-E 序列(即 SEQ ID NO: 4 的 RNA 等同物)具有至少 90%、至少 95%、至少 98%、至少 99% 或 100% 同一性的 prM-E 序列。优选在相当于 SEQ ID NO: 24 的 RNA 等同物的 524、736、1619 和 2055 位的所述 prM-E 序列内的位置上的核苷酸不突变(即在这些位置上保持出于在 SEQ ID NO: 24 的 RNA 等同物中的核苷酸)。

[0146] 有利的是,是用于本发明的疫苗组合物中的嵌合登革热病毒的血清型 2 的登革热抗原(例如与上文和本文其它部分描述的血清型 1、3 和 4 的登革热抗原(例如是 YF/ 登革热嵌合登革热病毒的血清型 1、3 和 4 的登革热抗原)联用)包含与 MD1280 毒株的 prM-E 序列(即 SEQ ID NO: 7 的 RNA 等同物)具有至少 90%、至少 95%、至少 98%、至少 99% 或 100% 同一性的 prM-E 序列。

[0147] 如本文所述,本发明的组合物可有利地包含选自以下的登革热抗原 :(a) 活的减毒登革热病毒 ;(b) 灭活的登革热病毒 ;(c) 活的减毒或灭活的嵌合登革热病毒和 (d) (a)-(c) 的两种或更多种的组合,其中所述登革热抗原包含选自 SEQ ID NO: 4、SEQ ID NO: 5、SEQ ID NO: 6、SEQ ID NO: 7 和 SEQ ID NO: 1 的核苷酸序列。

[0148] 如本文所述,本发明的组合物可有利地包含选自以下的登革热抗原 :(a) 活的减毒登革热病毒 ;(b) 灭活的登革热病毒 ;(c) 活的减毒或灭活的嵌合登革热病毒和 (d) (a)-(c) 的两种或更多种的组合,其中所述登革热抗原包含编码本文所述的 M 和 E 序列的核苷酸序列。

[0149] 例如本发明的组合物,例如用于本发明的保护方法的组合物,可有利地包含血清型 1、2、3 和 4 的登革热抗原的下列组合的任一种 :i) CYD-1、CYD-LAV、CYD-3 和 CYD-4 ;ii) CYD-1、CYD-BID、CYD-3 和 CYD-4 ;(iii) CYD-1、CYD-PR、CYD-3 和 CYD-4 或 (iv) CYD-1、CYD-MD、CYD-3 和 CYD-4。本发明的组合物还可有利地包含登革热抗原的下列组合 :i) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、VDV2、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原。例如,本发明的组合物可有利地包含 CYD-1、VDV-2、

CYD-3 和 CYD-4。如本文所述，本发明的组合物可有利地包含血清型 2 的登革热抗原，其包含 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列。如本文所述，本发明的组合物可有利地包含血清型 2 的登革热抗原，其包含与 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列具有至少 90% 同一性的序列。例如，所述序列可与 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列有至少 91%、至少 92%、至少 93%、至少 94%、至少 95%、至少 96%、至少 97%、至少 98% 或至少 99% 同一性。

[0150] 用于本发明的疫苗组合物，例如用于本发明方法的疫苗组合物优选包含是疫苗登革热病毒的登革热抗原。所述疫苗登革热病毒包括例如灭活病毒、活的减毒病毒和活的减毒嵌合登革热病毒。优选疫苗登革热病毒是活的减毒嵌合登革热病毒。优选本发明的活的减毒嵌合登革热病毒包含来自登革热病毒的一种或多种蛋白和来自不同黄病毒的一种或多种蛋白。有利的是，所述不同的黄病毒是黄热病病毒，例如毒株 YF 17D 的黄热病病毒。优选本发明的嵌合登革热病毒包含登革热病毒的 prM-E 氨基酸序列，例如本发明的嵌合登革热病毒包含其 prM-E 序列被登革热病毒的 prM-E 序列置换的黄热病病毒基因组。有利的是，本发明的疫苗组合物，例如用于本发明方法的疫苗组合物包含 CYD-1、CYD-2、CYD-3 和 CYD-4。本发明的组合物可有利地包含登革热抗原的下列组合的任一种 :i) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-LAV 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的嵌合登革热病毒和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ;ii) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-BID 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ;(iii) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-PR 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原 ;(iv) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、包含 CYD-MD 的 prM 和 E 序列的登革热抗原、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原。例如，本发明的组合物还可有利地包含登革热抗原的下列组合的任一种 :i) CYD-1、CYD-LAV、CYD-3 和 CYD-4 ; ii) CYD-1、CYD-BID、CYD-3 和 CYD-4 ;(iii) CYD-1、CYD-PR、CYD-3 和 CYD-4 或 (iv) CYD-1、CYD-MD、CYD-3 和 CYD-4。本发明的组合物还可有利地包含登革热抗原的下列组合 :i) 包含 CYD-1 的 prM 和 E 序列的登革热抗原、VDV2、包含 CYD-3 的 prM 和 E 序列的登革热抗原和包含 CYD-4 的 prM 和 E 序列的登革热抗原。例如，本发明的组合物可有利地包含 CYD-1、VDV-2、CYD-3 和 CYD-4。如本文所述，本发明的组合物可有利地包含血清型 2 的登革热抗原，其包含 CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11) 或 SEQ ID NO: 2 的 prM-E 序列。有利的是，本发明的疫苗组合物，例如血清型 2 的嵌合登革热病毒可包含与来自实施例中描述的血清型 2 毒株 LAV-2、BID-V585、PR/DB023 或 MD1280 的 prM-E 序列有至少 90%、至少 95%、至少 98% 或至少 99% 同一性的 prM-E 序列或可包含与 SEQ ID NO: 2 所示 prM-E 序列有至少 90%、至少 95%、至少 98% 或至少 99% 同一性的 prM-E 序列。有利的是，疫苗组合物，例如用于本发明方法的血清型 2 的嵌合登革热病毒可包含来自血清型 2 毒株 LAV-2、BID-V585、PR/DB023 或 MD1280 的 prM-E 序列或来

自实施例中描述的SEQ ID NO: 2的prM-E序列。当所述嵌合登革热病毒的受体基因组骨架来源于YF-VAX®时，所述毒株在本文称为CYD-LAV、CYD-BID、CYD-PR和CYD-MD。包含使用血清型2毒株LAV-2 (SEQ ID NO: 8)、BID-V585 (SEQ ID NO: 9)、PR/DB023 (SEQ ID NO: 10)、MD1280 (SEQ ID NO: 11)或SEQ ID NO: 2的prM-E序列产生或使用与来自血清型2毒株LAV-2、BID-V585、PR/DB023、MD1280的prM-E序列或来自SEQ ID NO: 2的prM-E序列具有至少90%、至少95%、至少98%或至少99%同一性的prM-E序列产生的血清型2的嵌合登革热病毒的本发明的疫苗组合物可有利地与CYD-1、CYD-3和CYD-4组合用于本发明的疫苗组合物中。如本文所述，本发明的组合物可有利地包含血清型2的登革热抗原，其包含与CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11)或SEQ ID NO: 2的prM-E序列具有至少90%同一性的序列。例如，所述序列可与CYD-LAV (SEQ ID NO: 8)、CYD-BID (SEQ ID NO: 9)、CYD-PR (SEQ ID NO: 10)、CYD-MD (SEQ ID NO: 11)或SEQ ID NO: 2的prM-E序列有至少91%、至少92%、至少93%、至少94%、至少95%、至少96%、至少97%、至少98%或至少99%同一性。

[0151] 待给予的本发明的疫苗登革热病毒的确切量可随待接种的患者的年龄和体重、给药频率以及组合物中的其它成分（例如佐剂）而变化。本发明的疫苗组合物中所包含的血清型1-4各自的活的减毒登革热病毒的量的范围为约10^3-约10^7 $CCID_{50}$。一般而言，本发明的疫苗组合物中所包含的血清型1-4各自的活的减毒登革热病毒的量的范围为约10^3-约10^6 $CCID_{50}$，例如范围为约5 x 10^3-约5 x 10^5 $CCID_{50}$、例如范围为约1 x 10^4-约1 x 10^5 $CCID_{50}$、例如约10^5 $CCID_{50}$。本发明的疫苗组合物中所包含的血清型1-4各自的活的减毒登革热病毒的量的范围还可为约10^4-约10^7 $CCID_{50}$，例如约10^6 $CCID_{50}$。本发明的四价组合物中所包含的血清型1-4各自的活的减毒登革热病毒的量可相等。例如本发明的四价组合物可包含约10^5 $CCID_{50}$的血清型1-4各自的活的减毒登革热病毒。或者，本发明的四价组合物可包含约10^6 $CCID_{50}$的血清型1-4各自的活的减毒登革热病毒。一般而言，本发明的组合物中所包含的血清型1-4各自的灭活的登革热病毒的量的范围为约10^4-约10^8 $CCID_{50}$当量，优选范围为约5 x 10^4-约5 x 10^7 $CCID_{50}$当量，优选范围为约1 x 10^4-约1 x 10^6 $CCID_{50}$当量，优选约10^5 $CCID_{50}$当量。一般而言，组合物中所包含的血清型1-4各自的VLP的量的范围为约100ng-约100μg VLP，优选范围为约100ng-约50μg、优选范围为约100ng-约20μg、优选约1μg-10μg。VLP的量可通过ELISA测量。有利的是，本发明的疫苗组合物包含有效量的本文定义的登革热抗原。

[0152] 本发明的疫苗组合物还可包含药学上可接受的载体或赋形剂。本发明的药学上可接受的载体或赋形剂意指任何溶剂或分散介质等，常用于药物和疫苗的制备中以提高活性剂的稳定性、无菌度和递送能力，且在人中不产生任何副反应，例如变态反应。根据所选的药物形式、给药方法和途径选择赋形剂。合适的赋形剂和有关药物制剂的要求描述于“Remington's Pharmaceutical Sciences”（第19版，A.R. Gennaro编辑，Mack Publishing Co., Easton, PA (1995)）。药学上可接受的赋形剂的具体实例包括水、磷酸缓冲盐水(PBS)溶液和0.3%甘氨酸溶液。本发明的疫苗组合物可有利地包含0.4%盐水和2.5%人血清白蛋白(HSA)。

[0153] 需要时，用于本发明方法的疫苗组合物可任选含有药学上可接受的辅助物质以接近生理条件，例如pH调节剂和缓冲剂、张力调节剂、润湿剂等，例如乙酸钠、乳酸钠、氯化

钠、氯化钾、氯化钙、单月桂山梨坦、油酸三乙醇胺、人血清白蛋白、必需氨基酸、非必需氨基酸、L- 精氨酸盐酸盐、蔗糖、无水 D- 海藻糖 (D-trehalose dehydrate)、山梨糖醇、三 (羟甲基) 氨基甲烷和 / 或脲。另外，疫苗组合物可任选包含药学上可接受的添加剂，包括例如稀释剂、粘合剂、稳定剂和防腐剂。优选的稳定剂描述于 WO 2010/003670。

[0154] 本发明的疫苗组合物可包含是登革热免疫蛋白质的登革热抗原。本文所用登革热免疫蛋白质是登革热包膜 (E) 蛋白或其衍生物或片段，当给予免疫活性受试者时诱导针对血清型 1、2、3 或 4 的登革热病毒的中和抗体。登革热免疫蛋白质包括登革热 E 蛋白的天然和衍生化形式，包括其化学缀合物、免疫片段和融合蛋白。

[0155] 登革热免疫蛋白质或其衍生物或片段可与载体分子缀合。所述缀合可通过化学缀合技术或通过包含登革热免疫蛋白质或其衍生物或片段和载体分子的融合蛋白的重组表达来实现。可用于制备缀合物的载体分子的实例包括白喉类毒素、破伤风类毒素、破伤风毒素的片段 C、包括 CRM 197、CRM 176、CRM228、CRM 45、CRM 9、CRM 45、CRM 102、CRM 103 和 CRM 107 在内的白喉毒素的突变体、肺炎球菌溶血素、OMPC、热休克蛋白、百日咳蛋白、肺炎球菌表面蛋白 PspA 或艰难梭菌 (*Clostridium difficile*) 的毒素 A 或 B。

[0156] 本发明的疫苗组合物可包含一种或多种佐剂以提高登革热抗原的免疫原性。本领域技术人员应能够选择在本发明的情况下是合适的佐剂。佐剂优选用于包含灭活病毒或 VLP 或登革热结构蛋白的本发明的疫苗组合物。佐剂可用于包含活的减毒病毒的本发明的疫苗组合物，只要所述佐剂不影响复制。

[0157] 合适的佐剂包括铝盐例如氢氧化铝凝胶、磷酸铝或明矾，但还可以是钙、镁、铁或锌的盐。其它合适的佐剂包括酰化酪氨酸或酰化糖、阳离子或阴离子衍生化糖或聚磷腈的不溶性混悬液。或者，佐剂可以是水包油乳液佐剂 (EP 0 399 843B)，以及水包油乳剂和其它活性剂的组合 (WO 95/17210、WO 98/56414、WO 99/12565 和 WO 99/11241)。描述了其它油乳液佐剂，例如油包水乳剂 (美国专利号 5,422,109、EP 0 480 982 B2) 和水包油包水乳剂 (美国专利号 5,424,067、EP 0 480 981 B)。所述佐剂的实例包括 MF59、AF03 (WO 2007/006939)、AF04 (WO 2007/080308)、AF05、AF06 及其衍生物。佐剂还可以是皂甙、脂质 A 或其衍生物、免疫刺激性寡核苷酸、烷基磷酸葡糖酰胺 (alkyl glucosamide phosphate)、水包油乳液或其组合。皂甙的实例包括 Quil A 和其纯化的片段，例如 QS7 和 QS21。

[0158] 本领域技术人员认识到，适宜配制与预定给药途径相容的本发明的疫苗组合物。合适的给药途径的实例包括例如肌内、经皮、皮下、鼻内、口服或皮内。有利的是，给药途径为皮下。

[0159] 可使用常规皮下注射器或安全注射器例如市购可获自 Becton Dickinson Corporation (Franklin Lakes, NJ, USA) 的注射器或射流注射器给予本发明的疫苗组合物。对于皮内给药，可使用利用 Mantoux 技术的常规皮下注射器或可使用专业的皮内递送装置例如 BD Soluvia(TM) 显微注射系统 (Becton Dickinson Corporation, Franklin Lakes, NJ, USA)。

[0160] 所给予的本发明的疫苗组合物的体积将取决于给药方法。在皮下注射的情况下，体积一般介于 0.1 和 1.0 ml 之间，优选约 0.5 ml。

[0161] 任选可在初次免疫后 (即在给予初始免疫方案排定的最后剂量的给药之后) 例如介于 6 个月和 10 年之间，例如 6 个月、1 年、3 年、5 年或 10 年，使用本发明的疫苗组合物的

加强给药。

[0162] 按照一个实施方案，本发明还提供包含本发明的疫苗组合物和所述疫苗组合物在保护人类受试者免于登革热病的方法中的用法说明的药盒。药盒可包含本文考虑的任何疫苗组合物的至少 1 剂（通常在注射器中）。按照一个实施方案，药盒可包含本文所述任何疫苗组合物的多剂量制剂（通常在小瓶中）。药盒还包含提及所述疫苗组合物用于预防登革热病的用途或所述疫苗用于预防登革热病的用途的活页。活页还可提及接种方案和待接种的受试者人群。

[0163] 可以多种方法测量本发明的疫苗组合物在降低登革热病的可能性或严重程度方面的功效。例如通过在给予至少 1 剂所述疫苗组合物后（例如在给予 1、2 或 3 剂所述疫苗组合物后），测量接受所述疫苗组合物的受试者组中的以下方面，来计算本发明的疫苗组合物在降低有症状的病毒学上已证实的登革热病的可能性或严重程度上的功效：

(i) 由任何血清型的登革热病毒引起的有症状的病毒学上已证实的登革热病例的百分比；

(ii) 由任何血清型的登革热病毒引起的病毒学上已证实的重度登革热病例的百分比；

(iii) 由任何血清型的登革热病毒引起的 I-IV 级登革出血热病例的百分比；

(iv) 由任何血清型的登革热病毒引起的 I 级 DHF 病例的百分比；

(v) 由任何血清型的登革热病毒引起的 II 级 DHF 病例的百分比；

(vi) 由任何血清型的登革热病毒引起的 III 级 DHF 病例的百分比；

(vii) 由任何血清型的登革热病毒引起的 IV 级 DHF 病例的百分比；

(viii) 由任何血清型的登革热病毒引起的住院的病毒学上已证实的登革热的年发生率；和 / 或

(ix) 由任何血清型的登革热病毒引起的有症状的病毒学上已证实的登革热病例的住院时间长度；

并对所述测量与未接受所述疫苗组合物的受试者的对照组的等同测量进行比较，其中在所述两组中的受试者居住在登革热地方病区。当与未接种受试者的对照组比较时，接种受试者组中 (i)-(ix) 任一个或多个的统计显著性降低表明本发明的疫苗组合物的功效。在一个优选的实施方案中，通过上述测量的一个或多个的统计显著性降低，来证实本发明的疫苗组合物的功效，其中 DHF 病例或登革热病例由血清型 1、3 或 4 的登革热病毒引起。

[0164] 还可通过在给予至少 1 剂所述疫苗组合物后（例如在给予 1、2 或 3 剂所述疫苗组合物后），测量接受所述疫苗组合物且已发生病毒学上已证实的登革热病的受试者组的以下方面，来计算本发明的疫苗组合物在降低登革热病的严重程度或可能性中的功效：

(i) 发热的平均持续时间和 / 或强度；

(ii) 由血细胞比容的变化限定的血浆渗漏的平均值；

(iii) 血小板减少（血小板计数）的平均值；和 / 或

(iv) 包括丙氨酸氨基转移酶 (ALT) 和天冬氨酸氨基转移酶 (AST) 在内的肝酶水平的平均值；

并对所述测量与未接受所述疫苗组合物且已发生病毒学上已证实的登革热病的受试者的对照组的等同测量进行比较。当与已发生病毒学上已证实的登革热病的受试者的对照

组比较时，已发生病毒学上已证实的登革热病的接种受试者组中 (i)-(v) 任一个或多个的统计显著性降低表明本发明的疫苗组合物在降低登革热病的严重程度或可能性中的功效。

[0165] 通常，例如通过实施例 1 所述方法 ($VE=100*(1-ID_{CYD}/ID_{对照})$，其中 ID 为各组中的发病密度（即患有病毒学上已证实的登革热的人类受试者的数目除以有风险的人 - 年的数目 (number of person-years at risk))) 测量的，本发明针对登革热病的保护方法的功效为至少 50%、优选至少 60%，其中所述登革热病由血清型 1、3 或 4 引起。针对血清型 3 或 4 引起的登革热病的保护方法的功效有利地为至少 70%、优选 80%。针对由血清型 4 引起的登革热病的保护方法的功效有利地为至少 90%。

[0166] 2 个氨基酸序列或 2 个核苷酸序列的百分比同一性通过标准比对算法确定，例如描述于 Altschul 等 (1990) J. Mol. Biol., 215: 403-410 的基础局部比对工具 (Basic Local Alignment Tool，BLAST)；Needleman 等 (1970) J. Mol. Biol., 48: 444-453 的算法；Meyers 等 (1988) Comput. Appl. Biosci., 4: 11-17 或 Tatusova 等 (1999) FEMS Microbiol. Lett., 174: 247-250 等的算法。将所述算法整合到 BLASTN、BLASTP 和“BLAST 2 Sequences”程序中（参见 www.ncbi.nim.nih.gov/BLAST）。当应用所述程序时，可使用默认参数。例如，对于核苷酸序列，可使用“BLAST 2 Sequences”的下列设置：程序 BLASTN，匹配奖励 2，错配罚分 -2，开放空位 (open gap) 和延长空位罚分分别为 5 和 2，空位 x~ 衰减 50，预期 10，字长 11，筛选 (filter) ON。对于氨基酸序列，可使用的下列设置“BLAST 2 Sequences”：程序 BLASTP，矩阵 BLOSUM62，开放空位 (open gap) 和延长空位罚分分别为 11 和 1，空位 x~ 衰减 50，预期 10，字长 3，筛选 (filter) ON。

[0167] 要了解，本文公开的本发明的各种特征和优选的实施方案可以组合在一起。

[0168] 在本申请中，引用了各种参考资料。这些参考资料的公开内容在此通过引用结合到本公开内容中。

[0169] 将通过下列实施例对本发明作进一步说明。然而应了解，本发明由权利要求书限定，且这些实施例仅作为本发明的说明给出，并不以任何方式构成对其的限制。

实施例

[0170] 实施例 1：在泰国对接种包含 Chimerivax™ DEN-1、DEN-2、DEN-3 和 DEN-4 的四价登革热疫苗 (TDV) 组合物的患者的 1 年随访

方法

研究设计和参与者

针对病毒学上已证实的登革热病，对四价 Chimerivax™ 疫苗（即包含由 DEN1 PUO359 (TYP 1 140) 的 prM 和 E 序列产生的特定 CYD-1 毒株、由 DEN2 PUO218 的 prM 和 E 序列产生的特定 CYD-2 毒株、由 DEN3 PaH881/88 的 prM 和 E 序列产生的特定 CYD-3 毒株和由 DEN4 1228 (TVP 980) 的 prM 和 E 序列产生的特定 CYD-4 毒株的四价疫苗，参见 WO 03/101397 和 Guy 等, Vaccine (2011), 29(42): 7229-41) 的功效进行了观察者不知情的随机对照单中心 IIb 期试验。招募 4002 名根据病史和身体检查健康状态良好的年龄为 4-11 岁的学童进入试验。研究在 Ratchaburi Regional Hospital (RRH), Ratchaburi province, Muang district, Thailand 进行。不包括招募时有急性发热疾病的儿童、有先天性或获得性免疫缺陷的儿童和接受免疫抑制疗法或其它抑制性治疗 (prohibited treatment) 或疫苗的儿

童。参与者以 2:1 随机分配，在第 0、6 和 12 个月时接受 3 剂登革热疫苗或对照制品。

[0171] 制品

按以下文献所述，产生每种嵌合病毒并在 Vero 细胞上培养：WO 03/10197；Guy 等，Hum. Vaccines (2010) 6 (9): 696；Guy等, Vaccine (2010) 28: 632；Guirakhoo等, J. Virol. (2000) 74: 5477；Guirakhoo 等, J. Virol. (2001) 75 (16): 7290；Guirakhoo 等, Virol. (June 20, 2002) 298: 146；以及Guirakhoo等, J. Virol. (2004) 78 (9): 4761。

[0172] 疫苗以冻干粉提供（之前保存在 2℃和 8℃之间的温度下），用 0.5 mL 注射用溶剂（含有 2.5% 人血清白蛋白的 0.4% NaCl）复溶。复溶时，各 0.5 mL 剂量的疫苗含有 5 ± 1 $\log_{10}$ $CCID_{50}$的各嵌合登革热血清型 (1、2、3 和 4) 和赋形剂（必需氨基酸、非必需氨基酸、L- 精氨酸盐酸盐、蔗糖、无水 D- 海藻糖、山梨糖醇、三（羟甲基）氨基乙烷和脲）。对照制品是灭活的狂犬病疫苗 (Verorab®, Sanofi Pasteur, Lyon France)，用于随机分配到对照组的前 50 名儿童的第 1 次注射，0•9% NaCl 盐水安慰剂用于所有其它注射。所有制品皮下注射到上臂三角肌区。

[0173] 评价

根据学期期间学校登记对旷课的每日监督（接着给旷课者打电话和家访），且整个假期每周两次家访、打电话或移动电话短信，主动监视所有儿童以检测急性发热疾病。在发热疾病（定义为相隔至少 4 小时两次温度读数≥ 37.5℃的疾病）的任何情况下，要求父母带他们的孩子到 RRH 诊断和治疗。监视系统也捕捉在 RRH 的自发会诊 (spontaneous consultation)。被≥ 14 天的无症状时间间隔分隔开的连续发热发作被视为单独发作。在发生时（即在发热开始后不多于 7 天收集的急性期样品）和 7-14 天后（恢复期样品）收集配对血清样品，并送往 Sanofi Pasteur's Global Clinical Immunology (GCI) 实验室 (Swiftwater, PA, USA) 和送往疫苗开发中心 (Centre for Vaccine Development) (CVD, Mahidol University, Thailand)。采用初始 RT-PCR 测定法，针对黄病毒的存在筛选急性期样品，该测定法检测任何黄病毒的存在（使用包含高度保守的黄病毒序列的引物）。如本文所述，用血清型特异性定量 RT-PCR 针对野生型登革热病毒测试阳性样品。平行地，使用商品化 ELIS 药盒 (Platelia™, Bio-Rad Laboratories, Marnes-La-Coquette, France)，针对登革热 NS1 抗原的存在测试所有急性期样品。登革热病的病毒学上已证实的发作定义为在血清型特异性 RT-PCR 或 NS1 抗原 ELISA 中为阳性结果。

[0174] 保持主动监视直到在第 3 次接种后跟踪各参与者至少 13 个月且直到独立数据监测委员会 (Independent Data Monitoring Committee, IDMC) 证实在符合方案 (per-protocol, PP) 人群中已发生≥ 27 例病例。

[0175] 在最后一次接种后，记录所有严重的不良事件 (SAE) 直到第 6 个月，再之后记录任何致死 SAE 或疫苗相关 SAE。

[0176] 在 RRH，在前 300 名入选儿童中，评价了在招募时和在每次注射后的 28 天收集的血清中的登革热免疫应答。在第 3 次注射后第 28 天，还前瞻性地收集了所有参与者的血清以评价从该时间点起发生的病毒学上已证实的登革热的儿童的免疫应答。血清被送往 GCI 以采用本文所述蚀斑减少中和试验 ($PRNT_{50}$) 测量针对 CYD 亲代登革热病毒的血清型特异性中和抗体滴度。测定的定量限为 10 (1/dil)。低于该值的样品被指定为滴度 5，并被视为血

清反应阴性。

[0177] 统计分析

主要目的是按照以下等式，在按计划招募和接种的儿童中，确定针对在第 3 次接种后超过 28 天发生的有症状的病毒学上已证实的登革热病例的疫苗功效 (VE)：VE =100*(1-$ID_{CYD}/ID_{对照}$)，其中 ID 为各组中的发病密度（即患有病毒学上已证实的登革热的儿童数除以有风险的人－年的数目）。其假定发病率为 1.3%，真实 VE 为 70%，在第 3 次接种后最少跟踪时间为 1 年，且符合方案 (PP) 受试者退出率 (attrition rate) 为 7.5%/ 年，需要以 2:1 比率分配到登革热疫苗或对照的 4002 名受试者以超过 82% 效能和 95% 置信度来证实 VE 非零。分析基于应用以下精确方法 (Exact method) 计算的 VE 的双侧 95% 置信区间 (CI)：(Breslow NE, Day NE. Statistical Methods in Cancer Research, 第 II 卷 – The Design and Analysis of Cohort Studies. International Agency for Research on Cancer (IARC 科技出版号 82), Lyon, France)。对 PP 人群进行了主要分析，所述 PP 人群即满足招募标准的人，其正确地在第 0、6（±15 天）和 12（±30 天）天接受了所有 3 剂指定疫苗，且对其组分配不是公开的。在接受 3 次注射的人中，对功效的全分析集重复了该分析。作为第二目的，在完成 3 剂接种方案前，测定针对登革热的 VE。在揭盲后限定的分析中，分别对针对各血清型的 VE 进行了研究。使用 95%CI，对安全性和免疫原性终点的研究是描述性的。

[0178] 结果

4002 名招募的儿童中，95.9% 完成接种，91.8% 包括在功效的符合方案 (PP) 分析集中。疫苗和对照组的年龄和性别相当。对于针对登革热或 JEV 的抗体，在基线采样的超过 90% 的样品为阳性。

[0179] 功效

在研究期间，131 例登革热病例 (131 名儿童有 136 次发作）是病毒学上已证实的。当此当中，在 PP 人群中，77 例发生在第 3 次注射后超过 28 天，并包括在主要分析中：45 例发生在疫苗组中的有风险的 2522 人－年期间，而 32 例发生在对照组中的有风险的 1251 人－年期间。相应的疫苗功效为 30・2% (95%CI：－ 13・4-56・6)。该发现在全分析集中被证实（参见下表 1)。至少 1 次注射后的功效为 33・4% (95%CI：4・1-53・5)，在至少 2 次注射后为 35・3% (95%CI：3・3-56・5)。

[0180] 表 1：血清型特异性和 CYD四价登革热疫苗针对病毒学上已证实的登革热病的总体功效

	登革热疫苗		对照		功效	
	有风险的人-年	病例或发作*	有风险的人-年	病例或发作*	%	(95% CI)
3 注射后>28 天 (符合方案分析)						
病例	2522	45	1251	32	30·2	(−13·4-56·6)
血清型 1 发作	2536	9	1251	10	55·6	(-21·6-84·0)
血清型 2 发作	2510	31	1250	17	9·2	(-75·3-51·3)
血清型 3 发作	2541	1	1257	2	75·3	(-375·0-99·6)
血清型 4 发作	2542	0	1263	4	100	(24·8-100)
NS1 抗原阳性仅发作	2542	4	1265	0	ND	ND
3 注射后>28 天 (全分析集)						
病例	2620	46	1307	34	32·5	(−8·5-57·6)
血清型 1 发作	2633	9	1308	10	55·3	(−22·5-83·9)
血清型 2 发作	2608	32	1307	19	15·6	(−57·6-53·6)
血清型 3 发作	2638	1	1312	2	75·1	(−378-99·6)
血清型 4 发作	2641	0	1320	4	100	(−24·3-100)
NS1 抗原阳性仅发作	2640	4	1322	0	ND	ND
至少 1 次注射后>28 天 (全分析集)						
病例	5089	75	2532	56	33·4	(4·1-53·5)
血清型 1 发作	5139	14	2564	18	61·2	(17·4-82·1)
血清型 2 发作	5107	51	2560	26	1·7	(−64·3-39·8)
血清型 3 发作	5144	4	2565	10	80·1	(30·9-95·4)
血清型 4 发作	5149	1	2577	5	90·0	(10·5-99·8)
NS1 抗原阳性仅发作	5147	5	2579	1	−150·5	(−11750-72·0)

数据是数值，除其中有说明以外。ND：未测定。* “病例”定义为通过血清型特异性 PCR 或 NS1 抗原 ELISA 的病毒学上已证实的登革热的首次发作。计算血清型特异性功效包括该血清型的全部发作；因此研究期间有两次病毒学上证实的登革热发作的 5 名儿童两次包括在血清型特异性分析中。

事后分析显示血清型的不同功效（参见表 1）。与针对 DENV2 的 1.7% 相比，在至少 1 次注射后针对 DENV1、DENV3 和 DENV4 的功效的范围为 61·2%-90·0%。与针对 DENV2 的 15.6% 相比，在 3 次注射后针对 DENV1、DENV3 和 DENV4 的功效的范围为 55.3%-100%。

[0181] 在获得病毒学上已证实的登革热的那些受试者中，当与对照组相比时，在接种疫苗组中观察到年住院发生率统计显著性降低。3 剂后的相对风险 (RR) 为 0.523 （参见表 2）。

[0182] 表 2:试验期间住院的病毒学上已证实的登革热的发生率

期间	CYD 登革热疫苗组 (N=2666) M	病例	年发病率 (95%CI)	n 发生	对照组 (N=1331) M	病例	年发病率 (95%CI)	n 发生	相对风险 RR	(95%CI)
第 1 年	2666	8	0.3 (0.1; 0.6)	8	1331	7	0.5 (0.2; 1.1)	7	0.571	(0.181, 1.85)
第 2 年	2557	24	0.9 (0.5; 1.3)	24	1282	23	1.7 (1.0; 2.5)	23	0.523	(0.283, 0.970)

第 1 年 = 第 0 天至第 3 次注射 ;第 2 年 = 第 3 次注射到活跃期结束

表 3:按血清型区分的住院率

	接受疫苗者组(%)	对照组(%)
血清型 1	8/14 (57.1)	9/18 (50%)
血清型 2	20/52 (38.5)	15/27 (55.6)
血清型 3	1/4 (25)	3/11 (27.3)
血清型 4	0/1	2/5 (40)
无血清型 NS1 +ve	3/5 (60)	1/1 (100)
共计	32/76 (42.1)	30/62 (48.4)

免疫原性

在符合方案分析集中,在疫苗组中,在第 3 次注射后第 28 天针对登革热血清型 1-4 的中和抗体的几何平均滴度 (GMT) 分别为 146 (95%CI :98・5-217)、310 (224-431)、405 (307-534) 和 155 (123-196)。在对照组中,这些值为 23・9 (14・0-40・9)、52・2 (26・8-102)、48・9 (25・5-93・9) 和 19・4 (11・6-32・2)。1 年后,血清型 1、2、3 和 4 的 GMT 分别为 76.5、122、94 和 153。

[0183] 安全性

在该期研究期间有 584 例 SAE :在疫苗组中报告了 366 例,占参与者的 11.8% (315/2666),在对照组中报告了 218 例,占参与者的 13.2% (176/1331)。在登革热组中无疫苗相关 SAE,在对照组中有 1 例。所观察到的 SAE 是与年龄组一致的医学病况,并显示在接种期后 7 和 28 天内无聚类 (clustering)。

[0184] 在接受疫苗者中作为突破发生的病毒学上已证实的登革热病例不比在对照组中发生的那些病例严重。

[0185] *试验中传播性野生型血清型 2毒株的 prM-E区域的序列*

测定了在试验中引起 DEN-2 病例的野生型血清型 2 毒株的 prM-E 区域的核苷酸和氨基酸序列。这些分别由以下 SEQ ID NO: 1 和 SEQ ID NO: 2 所示。试验中引起 DEN-2 病例的血清型 2 毒株的 E 和 M 氨基酸序列分别描述于 SEQ ID NO: 18 和 23。

[0186] >核苷酸序列 (SEQ ID NO: 1)

ttccatctaaccacacgcaacggagaaccacacatgatcgtcggtatacaggagaaagggaaaagtcttct
gttcaaaacagaggatggtgtgaacatgtgcaccctcatggctatggaccttggtgaattgtgtgaagacacaatc
acgtacaagtgtcctcttctcaggcagaatgagccagaagacatagactgttggtgcaactccacgtccacgtgggt
aacctatgggacctgtaccactacgggagaacataggagagaaaaaagatcagtggcactcgttccacatgtgggaa
tgggactggagacgcgaaccgaaacatggatgtcatcagaaggggcttggaaacatgcccagagaattgaaacttgg
atcctgagacatccaggcttcaccataatggcagcaatcctggcatacaccataggaacgacacatttccagagagt

cctgattttcatcctactgacagctgtcgctccttcaatgacaatgcgttgcataggaatatcaaatagagactttg
tagaaggggtttcaggaggaagttgggttgacatagtcttagaacatggaagctgtgtgacgacgatggcaaaaaac
aaaccaacattggatttcgaactgataaaaacggaagccaaacagcctgccaccctaaggaagtactgcatagaagc
aaaactaaccaacacaacaacagaatcccgttgcccaacacaaggggaacccagcctaaaagaagagcaggacaaga
ggttcgtctgcaaacactccatggtagacagaggatggggaaatggatgtggattatttggaaagggaggcattgtg
acctgtgctatgttcacatgcaaaaagaacatggaagggaaaatcgtgcaaccagaaaacttggaatacaccattgt
ggtaacacctcactcaggggaagagcatgcggtcggaaatgacacaggaaaacacggcaaggaaatcaaagtaacac
cacagagttccatcacagaagcagaactgacaggttatggcaccgtcacgatggagtgctccccgagaacaggcctc
gacttcaatgagatggtgttgctgcagatggaaaataaagcttggctggtgcataggcaatggtttctagacctgcc
attaccatggctgcccggagcggataaacaagaatcaaattggatacagaaagaaacattggtcactttcaaaaatc
cccatgcgaagaaacaggatgttgttgttttaggatcccaagaaggggccatgcatacagcactcacaggagccaca
gaaatccaaatgtcgtcaggaaacttgctcttcactggacatctcaagtgcaggctgagaatggacaagctacagct
taaaggaatgtcatactctatgtgcacaggaaagtttaaagttgtgaaggaaatagcagaaacacaacatggaacga
tagttatcagagtgcaatatgaaggggacggctctccatgtaaaattccttttgagataatggatttggaaaaaaga
tatgtcttaggccgcctgatcacagtcaacccaattgtaacagaaaaagacagcccagtcaacatagaagcagaacc
tccattcggagacagttacatcatcataggagtagagccgggacaactgaagctcaactggttcaagaaaggaagtt
ctatcggccaaatgtttgagacaacgatgagaggggcgaagagaatggccattttgggtgacacagcctgggacttc
ggatccctgggaggagtgtttacatctataggaaaagctctccaccaagtctttggagcgatctatggggctgcctt
cagtggggtttcatggaccatgaaaatcctcataggagtcattatcacatggataggaatgaactcacgcagcacct
cactgtctgtgtcactggtactggtgggaattgtgacactgtatttaggagtcatggtgcaggcc

>氨基酸序列(SEQ ID NO: 2)

FHLTTRNGEPHMIVGIQEKGKSLLFKTEDGVNMCTLMAMDLGELCEDTITYKCPLLRQNEPEDIDCWCNST
STWVTYGTCTTTGEHRREKRSVALVPHVGMGLETRTETWMSSEGAWKHAQRIETWILRHPGFTIMAAILAYTIGTT
HFQRVLIFILLTAVAPSMTMRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRK
YCIEAKLTNTTTESRCPTQGEPSLKEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENL
EYTIVVTPHSGEEHAVGNDTGKHGKEIKVTPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMENKAWLVHRQW
FLDLPLPWLPGADKQESNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRM
DKLQLKGMSYSMCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRYVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAI
YGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGIVTLYLGVMVQA

>氨基酸序列(SEQ ID NO: 18)

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTE
SRCPTQGEPSLKEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENLEYTIVVTPHSGE
EHAVGNDTGKHGKEIKVTPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGA
DKQESNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSM
CTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRYVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYI
IIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTM
KILIGVIITWIGMNSRSTSLSVSLVLVGIVTLYLGVMVQA

>氨基酸序列(SEQ ID NO: 23)

SVALVPHVGMGLETRTETWMSSEGAWKHAQRIETWILRHPGFTIMAAILAYTIGTTHFQRVLIFILLTAVAPS MT

讨论

该研究的主要结果是基于嵌合CYD病毒的针对登革热的安全有效的疫苗是可能的。针对DENV1、3和4的估计功效在与70%假设一致的范围内，并且在至少1次接种后是统计显著性的。未观察到在针对DENV2的与70%假设一致的范围内的功效。因为DENV2在该研究中是流行血清型(prevalent serotype)，在这种情况下，总体疫苗功效减弱。

[0187] 疫苗的安全性和反应原性特征(reactogenicity profile)良好，无疫苗相关SAE，并且在评审中在主动跟踪超过2600名疫苗接种者两年间所收集的AE和SAE期间，未鉴定到安全信号。因针对4种登革热血清型的不完全免疫应答所致与登革热病的发生率或严重程度潜在提高有关的理论安全顾虑之前妨碍了疫苗开发。在该试验中，在针对传播性DENV2病毒的不完全免疫应答存在下没有疾病增强是重要的和令人安慰的结果。例如，就例如发热持续时间等因素而言或就例如出血、血浆渗漏或血小板减少等登革热的经典临床征象而言，接受疫苗者中的病例与对照中的病例没有不同。此外，与试验期间任何点上的对照相比，接受疫苗者中的重度登革热不更常见。

[0188] 还证实了当与对照组相比时，在获得病毒学上已证实的登革热的那些受试者中，在接种疫苗组中观察到年住院发生率统计显著性降低。在获得血清型2的病毒学上已证实的登革热的那些受试者中观察到这种降低(参见表3)。

[0189] 有关DENV2所观察到的结果可通过多个起作用的因素来解释。例如，在试验中引起疾病的CYD2疫苗病毒和DENV2病毒之间存在可能的抗原错配。在1990年代，出现在东南亚的DENV2的亚洲1基因型，替代了之前占优势的亚洲/美洲病毒谱系。在E蛋白的结构域2中鉴定出的几个突变(E83，特别是E226和E228)暗示变化中的病毒适合度和抗原性。CYD2疫苗的供体野生型病毒(和用于PRNT50的攻击毒株)是1980年得自曼谷的临床分离株(Guirakhoo F等，J Virol 2000，74：5477-85)。虽然该病毒也归类为属于亚洲I基因型，但在该病毒(因此在CYD2)中的上述关键氨基酸残基对应于亚洲/美洲基因型的哪些(Hang等，PLoS Negl Trop Dis. 2010年7月20日；4(7)：e757)。

[0190] 另外，在CYD2疫苗的prM-E序列中有2个极端罕见的突变，其也可归因于错配的免疫应答。这些突变位于prM24和E251位处(Guirakhoo等，J. Virol. (2004) 78 (9)：4761)。

[0191] 针对DENV2所观察到结果与在$PRNT_{50}$测定法中不存在免疫原性没有关联。在接种后针对DENV2的中和抗体反应高于针对DENV1和DENV3的中和抗体反应。

[0192] 综上所述，本研究构成首次曾经证明，安全和有效的登革热疫苗是可能的，并代表了登革热疫苗开发中的重要里程碑。

[0193] 实施例2：血清型2的优化登革热疫苗毒株的鉴定

本实施例的目的是鉴定血清型2的登革热病毒毒株，其为产生针对血清型2的登革热病毒的优化登革热疫苗组合物提供基础，其中当用于本发明的方法时，与Chimerivax™ CYD-2相比，所述优化的登革热疫苗组合物提供改进的功效。

[0194] 用于确定世界性登革热2抗原的优化毒株选择的标准包括：(i)最近传播的毒株；(ii)亚洲和美洲毒株之间的平衡选择；(iii)优化毒株应具有这样的prM-E序列，其尽可

能地类似于计算出的总共有序列 (global consensus sequence),所述共有序列通过比对可获得的血清型 2 的登革热病毒的 prM-E 序列产生 ;(iv) 应避免预测影响抗体识别的氨基酸变异 ;(v) 应避免 prM 和 E 序列中特定位置上的稀有氨基酸,尤其是在 E 蛋白胞外域中 (在特定位置上的稀有氨基酸定义为在小于 15% 的比对序列中的该位置上出现的氨基酸) ;(vi) 优选一些之前实验室经验存在的优化毒株,和 (vii) 在四价组合物中导致平衡的免疫应答的登革热抗原。确定地方性登革热 2 抗原的优化毒株选择的标准 (即针对特定地区传播的野生型登革热病毒特别有效) 为标准 (i) 和 (vii)。

[0195] 方法

数据库

序列从国家生物技术信息中心 (National Center for Biotechnology Information,NCBI) 登革热病毒变异数据库检索 (www.ncbi.nlm.nih.gov/genomes/VirusVariation/Database/nph-select.cgi?tax_id=12637)。

[0196] *序列分析*

序列比对应用 MUSCLE 算法进行 (Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput (MUSCLE :具有高精确度和高通量的多序列比对). Nucleic Acids Res, 32(5):1792-1797)。

[0197] 序列比对输出在 Vector NTi 第 9 版,模块 AlignX (Invitrogen) 中产生。序列相似性检索应用 BLAST 算法进行 (Altschul, S. F., Gish, W., Miller, W., Myers, E. W. 和 Lipman, D. J. (1990) Basic local alignment search tool (基础局部比对搜索工具). J Mol Biol, 215(3):403-410)。

[0198] *prM-E序列的序列编号*

PrM-E 序列中所包括的亚序列可以不同方式编号 :(i) 总的 prM-E 蛋白序列从 1 位到 661 位编号,其 preM 蛋白质序列指定为 1 位到 90/91 位,M 蛋白序列指定为 91/92 位 -166 位,E 蛋白序列指定为 167 位 -661 位 ;(ii) prM 和 M 蛋白序列在一起编号,即从总序列的 1 位 -166 位,E 单独从 1 位 -495 位编号 ;(iii) prM、M 和 E 序列分别编号,即 prM 从 1 位 -90/91 位编号,M 从 1 位到 75/76 位编号,E 从 1 位到 495 位编号。

[0199] 结果

公共序列检索

所有可获得的登革热病毒血清型 2 全长 prM 和 E 蛋白序列自 NCBI 登革热数据库下载。序列下载发生在两个单独的时间 - 2010 年 10 月 4 日和 2011 年。在第一时间下载了 669 个序列,在第二时间下载了约 3200 个序列。

[0200] *总共有序列产生*

在各个时间,对全部所检索的蛋白质序列进行比对以产生血清型 2 登革热病毒的 prM 和 E 蛋白的总共有序列。根据定义,总共有序列是在各个位置上含有最频繁遇到的氨基酸的人工序列。2010 比对和 2011 比对的总共有序列只有 2 个氨基酸之差。在 2010 比对中,E 蛋白的总共有序列分别在 129 和 308 位含有异亮氨酸和缬氨酸 (参照 1-495 E 序列编号),相比之下,在 2011 比对中,E 蛋白的总共有序列分别在 129 和 308 位上含有缬氨酸和异亮氨酸 (参照 1-495 E 序列编号)。2010 和 2011 总共有序列中的差异通过如下事实解释 :在所述位置上含有缬氨酸或异亮氨酸的毒株各自的百分比接近 50%。因此如下表示 prM-E 序

列的总共有序列：

fhlttrngephmivgrqekgksllfktedgvnmctlmaidlgelcedtitykcpllrqnepedidcwcnst stwvtygtctttgehrrekrsvalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtt hfqralifilltavapsmtMRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRK YCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKXVQPENL EYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQW FLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRM DKLQLKGMSYSMCTGKFKZVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAI YGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA (SEQ ID NO: 3)

如下表示 E 序列的总共有序列：

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTESR CPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKXVQPENLEYTIVITPHSGEEHA VGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGADTQ GSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSMCTG KFKZVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYIIIG VEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTMKIL IGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA (SEQ ID NO: 12)

在上述序列中，总共有 prM 序列以小写字母表示，E 序列以大写字母表示。注明为 X (E 序列的 129 位）和 Z (E 序列的 308 位）的氨基酸位置各自独立地为 Val 或 Ile，即在这些位置上比对的氨基酸序列（包括 Val 或 Ile）的比例接近 50%。

[0201] *Chimerivax™ CYD2序列的少量氨基酸残基的确定和分析*

由含有以至少 5% 比对序列变化的所有氨基酸位置的总比对，建立可变氨基酸位置列表。另外，还鉴定了不匹配总共有序列的 Chimerivax™ CYD2 的 prM 和 E 蛋白序列的任何氨基酸。结果见表 4 (N.B.，在该表中，prM 和 M 蛋白序列共同编号，即从总序列的 1 位 -166 位编号，E 单独从 1 位 -495 位编号）。

[0202] 表 4:登革热病毒血清型 2可变残基和 CYD2比较

在 prM 和 E 序列中鉴定出总共 41 个氨基酸位置,其以至少 5% 的比对序列而不同于总共有序列和 / 或不同于 CYD2 中的 prM 和 E 蛋白的序列。CYD2 中 prM 和 E 蛋白序列的 10 个氨基酸位置不同于总共有序列 (E 中 5 个位置,M 中 2 个位置,其前体部分中 3 个,参见表 4)。10 个不同残基中有 5 个呈现接近 50:50 的变动分布 (variation distribution),表明了天然可变的位置。CYD2 prM-E 序列中仅 3 个位置以非常少的改变出现 (pr-24 Val、M-125 Ile 和 E-251 Phe)。

[0203] E 和 M 蛋白中改变的影响分析

为了进一步得到可变位置,还进一步分析了 E 蛋白胞外域 (氨基酸 1-395) (通过免疫系统进行血清中和的最重要的结构域) 的变化。

[0204] 利用可获自血清型 2 的登革热病毒 E 蛋白的可溶性胞外域已公布的 3D 结构的信息 (Modis,Y. 等 (2003) Proc Natl Acad Sci U S A,100(12) :6986-6991),重构了登革热病毒颗粒表面的 3D 模型。这允许微调来自 E 胞外域的各个氨基酸的可及性的评价,这进而与氨基酸变化的变动性水平和性质联用以评价 CYD2 变异对抗体识别的潜在影响。

[0205] 分析证实来自总共有序列的 Chimerivax™ CYD2 序列中的 2 个改变 (E 蛋白的 Val 141 和 Val 164) 完全埋在 3D 结构中,因此不能在病毒粒子表面与抗体直接相互作用。E 蛋白的 129 位是介于 Val (Chimerivax™ CYD2) 和 Ile (总共有序列) 之间的 50:50 可变氨基酸位置,且取代也是完全保守的变化。因此这些变动的潜在影响被视为非常有限。

[0206] E 蛋白中 203 位的变动 (Chimerivax™ CYD2 中的 Asn 和总共有序列中的 Asp) 可能具有影响 (完全暴露的残基,电荷的变化),但毒株间变动的分布接近 50:50,表明了天然可变的位置。

[0207] Chimerivax™ CYD2 的 E 蛋白的 251 位的变动 (Chimerivax™ CYD2 中的 Phe 和总共有序列中的 Val) 在检索的毒株中极罕见。所述变动对被抗体识别可能有一些影响,因为它罕见,极充分地暴露在病毒体的表面 (29%),且相当于非保守氨基酸变化。

[0208] 上述建模分析在 E 蛋白中鉴定出可能对抗体识别具有潜在影响的其它 2 个位置变动 (226 和 228 位),虽然 Chimerivax™ CYD2 在这些位置上没有不同于总共有序列。因此在鉴定优化血清型 2 毒株方面,对于世界性登革热 2 疫苗,优选避免在所述位置上与总共有序列的变动 (即 226 位的 Thr 和 228 位的甘氨酸)。

[0209] 虽不受理论束缚,但本发明人认为还可采用评分方法,评价登革热病毒序列中氨基酸变动的影响,该方法考虑多个相关因素。特别是该方法考虑了基因组位置的变动 (G)、氨基酸性质的变化 (B)、3D 作图 (M) 和在所述位置上的已知变体 (DB),其中按 G x B x M x DB 计算评分。0 分可归类为无预期影响,>0-10 的评分可归类为低预期影响,>10-25 的评分可归类为中等预期影响,>25 的评分可归类为高预期影响。

[0210] 如果氨基酸位于 prM/M 蛋白的 M 部分 (即 prM/M 序列的 92-166 位) 中或 E 蛋白的 396-495 位中,则基因组位置 (G) 评分为 0。如果氨基酸位于 prM/M 蛋白的 prM 部分 (即 prM/M 序列的 1-91 位) 中或 E 蛋白的 1-395 位中,则基因组位置评分为 1。

[0211] 按 B = 100 - [(Blosum95 评分 + 6) x 10] 计算与氨基酸性质的变化 (B) 有关的评分,其中不同氨基酸取代的 Blosum95 评分如下表 5 所示。

[0212] 表 5

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	*
A		-2	-2	-3	-1	-1	-1	-1	-3	-2	-2	-1	-2	-3	-1	1	0	-4	-3	-1	-3	-1	-1	-6
R	-2		-1	-3	-5	0	-1	-4	-1	-4	-3	2	-2	-4	-3	-2	-2	-4	-3	-4	-2	-1	-2	-6
N	-2	-1		1	-4	0	-1	-1	0	-4	-5	0	-3	-4	-3	0	-1	-5	-3	-4	4	-1	-2	-6
D	-3	-3	1		-5	-1	1	-2	-2	-5	-5	-2	-5	-5	-3	-1	-2	-6	-5	-5	4	0	-2	-6
C	-1	-5	-4	-5		-4	-6	-5	-5	-2	-3	-5	-3	-3	-5	-2	-2	-4	-4	-2	-4	-5	-3	-6
Q	-1	0	0	-1	-4		2	-3	1	-4	-3	1	-1	-4	-2	-1	-1	-3	-3	-3	-1	4	-1	-6
E	-1	-1	-1	1	-6	2		-3	-1	-4	-4	0	-3	-5	-2	-1	-2	-5	-4	-3	0	4	-2	-6
G	-1	-4	-1	-2	-5	-3	-3		-3	-6	-5	-3	-4	-5	-4	-1	-3	-5	-5	-5	-2	-3	-3	-6
H	-3	-1	0	-2	-5	1	-1	-3		-4	-4	-1	-3	-2	-3	-2	-2	-3	1	-4	-1	0	-2	-6
I	-2	-4	-4	-5	-2	-4	-4	-6	-4		1	-4	1	-1	-4	-3	-2	-4	-2	3	-5	-4	-2	-6
L	-2	-3	-5	-5	-3	-3	-4	-5	-4	1		-3	2	0	-4	-3	-2	-3	-2	0	-5	-4	-2	-6
K	-1	2	0	-2	-5	1	0	-3	-1	-4	-3		-2	-4	-2	-1	-1	-5	-3	-3	-1	0	-1	-6
M	-2	-2	-3	-5	-3	-1	-3	-4	-3	1	2	-2		-1	-3	-3	-1	-2	-3	0	-4	-2	-2	-6
F	-3	-4	-4	-5	-3	-4	-5	-5	-2	-1	0	-4	-1		-5	-3	-3	0	3	-2	-5	-4	-2	-6
P	-1	-3	-3	-3	-5	-2	-2	-4	-3	-4	-4	-2	-3	-5		-2	-2	-5	-5	-4	-3	-2	-3	-6
S	1	-2	0	-1	-2	-1	-1	-1	-2	-3	-3	-1	-3	-3	-2		1	-4	-3	-3	-1	-1	-1	-6
T	0	-2	-1	-2	-2	-1	-2	-3	-2	-2	-2	-1	-1	-3	-2	1		-4	-3	-1	-1	-2	-1	-6
W	-4	-4	-5	-6	-4	-3	-5	-5	-3	-4	-3	-5	-2	0	-5	-4	-4		2	-3	-6	-4	-4	-6
Y	-3	-3	-3	-5	-4	-3	-4	-5	1	-2	-2	-3	-3	3	-5	-3	-3	2		-3	-4	-4	-2	-6
V	-1	-4	-4	-5	-2	-3	-3	-5	-4	3	0	-3	0	-2	-4	-3	-1	-3	-3		-5	-3	-2	-6
B	-3	-2	4	4	-4	-1	0	-2	-1	-5	-5	-1	-4	-5	-3	-1	-1	-6	-4	-5		0	-2	-6
Z	-1	-1	-1	0	-5	4	4	-3	0	-4	-4	0	-2	-4	-2	-1	-2	-4	-4	-3	0		-1	-6
X	-1	-2	-2	-2	-3	-1	-2	-3	-2	-2	-2	-1	-2	-2	-3	-1	-1	-4	-2	-2	-2	-1		-6
*	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	-6	

B = Asx,Z = Glx,X = 任何,* = 终止

M 值取决于氨基酸是否位于 prM/E 界面。例如，对于如实施例 1 中使用的 CYD2，位于该界面的氨基酸为 prM 残基 6、7、39、40、46-54、56、59-65、67、74 和 77 和 E 残基 64-72、82-84、101-104、106-108 和 244-247。当氨基酸位于该界面时，M 等于 1。如果氨基酸不位于该界面，则 M = Y x SAS%。如果氨基酸位于“上”位（即指向外部环境），则 Y 为 1；如果氨基酸位于分子“侧”（即氨基酸既不指向外部环境也不指向衣壳），则 Y 为 0.5，如果氨基酸位于“下”位（即指向衣壳），则 Y 为 0。应用 Discovery Studio 3D 建模软件 (Accelrys, Inc., CA, USA) 产生溶剂可及性表面 % (SAS%) 值。

[0213] 当氨基酸取代导致在该取代位置上的氨基酸是存在于 GenBank 数据库 (http://www.ncbi.nlm.nih.gov) 中的登革热序列中该位置的最常见氨基酸时，DB 值为 0。当氨基酸取代导致在该取代位置的氨基酸以超过 5% 的存在于数据库中的登革热序列（但不是该位置上的最常见氨基酸）存在时，则 DB 值为 0.25。当氨基酸取代导致在该取代位置的氨基酸以小于 5% 的存在于数据库中的登革热序列（唯一取代除外）存在时，则 DB 值为 0.50。当取代氨基酸是唯一的，则 DB 值为 1。

[0214] 复制期间，病毒可获得导致氨基酸取代的突变。上述方法提供确定所述突变对突变病毒子代的作用的手段。

[0215] 优选的序列（即被视为令人满意地接近已鉴定的共有序列的序列）可具有：(i) 至多两个、优选一个或无高影响氨基酸取代；(ii) 至多 3 个、优选两个或一个或无中等影响氨基酸取代；和 / 或 (iii) 至多 5、4、3、2 或 1 个低影响氨基酸取代。

[0216] *优化的血清型 2 毒株的鉴定*

根据上述选择标准鉴定优化的血清型 2 毒株。

[0217] 进行了 BLAST 搜索以鉴定在所有可获得的序列中与 prM-E 总共有序列具有最接近的序列的毒株。未发现与 prM-E 总共有序列有 100% 同一性的序列，但最佳命中是得自毒株 BID-V585 (NCBI 蛋白质编号 ACA58343；基因组编号 EU529706；2006 年自波多黎各分离）的序列，该序列只显示与总共有序列在 91 位处的一个变动（总共有序列中的 Val 和 BID-V585 中的 Ile）。BID-V585 prM-E 序列与 Chimerivax™ CYD-2 prM-E 序列相比含有 13 个变动。

[0218] 进行了进一步的毒株选择以提供毒株起源的地理平衡。因此选择在 BLAST 分析中显示良好评分的最近分离的亚洲毒株（毒株 MD-1280；NCBI 蛋白质编号 CAR65175；基因组编号 FM21043；2004 年自越南分离）。尽管显示在 prM-E 中具有总共有序列的 6 个变动，但 6 个变动中的 3 个被鉴定为在超过 30% 的毒株中天然变化的多变位置。MD-1280 prM-E 序列含有与 Chimerivax™ CYD-2 prM-E 序列区分的 15 个变动。

[0219] 根据大量之前积累的有关毒株的经验进行进一步的毒株选择。它是 PDK53-16681 毒株，亦称为 LAV-2 毒株，一种来源于来自 Mahidol University 的登革热血清型 2 16681 毒株的活的减毒病毒 (NCBI 蛋白质编号 AAA73186；基因组编号 M84728；1964 年自泰国分离；Blok, J. 等 (1992)；Virology 187 (2)，573-590)。LAV-2 prM-E 序列含有与总共有序列区分的 10 个变动，与 Chimerivax™ CYD-2 prM-E 序列区分的 13 个变动。

[0220] 基于上述标准选择的另一个毒株是毒株 PR/DB023 (NCBI 蛋白质编号 AEN71248；基因组编号 JF804036；2007 年自波多黎各分离）。PR/DB023 prM-E 序列含有与总共有序列区分的 3 个变动，与 Chimerivax™ CYD-2 prM-E 序列区分的 13 个变动。

[0221] 所选择的毒株无一含有存在于 Chimerivax™ CYD-2 prM-E 序列的稀有氨基酸，即

在 prM-24 处的 Val、M-125 处的 Ile 和 E-251 处的 Phe。

[0222] 四个所选毒株的 PrM-E核苷酸序列

>LAV-2 prME核苷酸序列 (SEQ ID NO: 4)

ttccatttaaccacacgtaacggagaaccacacatgatcgtcagcagacaagagaaagggaaaagtcttct
gtttaaaacagaggttggcgtgaacatgtgtaccctcatggccatggaccttggtgaattgtgtgaagacacaatc
acgtacaagtgtccccttctcaggcagaatgagccagaagacatagactgttggtgcaactctacgtccacgtgggt
aacttatgggacgtgtaccaccatgggagaacatagaagagaaaaaagatcagtggcactcgttccacatgtgggaa
tgggactggagacacgaactgaaacatggatgtcatcagaaggggcctggaaacatgtccagagaattgaaacttgg
atcttgagacatccaggcttcaccatgatggcagcaatcctggcatacaccataggaacgacacatttccaaagagc
cctgattttcatcttactgacagctgtcactccttcaatgacaATGCGTTGCATAGGAATGTCAAATAGAGACTTTG
TGGAAGGGGTTTCAGGAGGAAGCTGGGTTGACATAGTCTTAGAACATGGAAGCTGTGTGACGACGATGGCAAAAAAC
AAACCAACATTGGATTTTGAACTGATAAAAACAGAAGCCAAACAGCCTGCCACCCTAAGGAAGTACTGTATAGAGGC
AAAGCTAACCAACACAACAACAGAATCTCGCTGCCCAACACAAGGGGAACCCAGCCTAAATGAAGAGCAGGACAAAA
GGTTCGTCTGCAAACACTCCATGGTAGACAGAGGATGGGGAAATGGATGTGGACTATTTGGAAAGGGAGGCATTGTG
ACCTGTGCTATGTTCAGATGCAAAAAGAACATGGAAGGAAAAGTTGTGCAACCAGAAAACTTGGAATACACCATTGT
GATAACACCTCACTCAGGGGAAGAGCATGCAGTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACAC
CACAGAGTTCCATCACAGAAGCAGAATTGACAGGTTATGGCACTGTCACAATGGAGTGCTCTCCAAGAACGGGCCTC
GACTTCAATGAGATGGTGTTGCTGCAGATGGAAAATAAAGCTTGGCTGGTGCACAGGCAATGGTTCCTAGACCTGCC
GTTACCATGGTTGCCCGGAGCGGACACACAAGGGTCAAATTGGATACAGAAAGAGACATTGGTCACTTTCAAAAATC
CCCATGCGAAGAAACAGGATGTTGTTGTTTTAGGATCCCAAGAAGGGGCCATGCACACAGCACTTACAGGGGCCACA
GAAATCCAAATGTCATCAGGAAACTTACTCTTCACAGGACATCTCAAGTGCAGGCTGAGAATGGACAAGCTACAGCT
CAAAGGAATGTCATACTCTATGTGCACAGGAAAGTTTAAAGTTGTGAAGGAAATAGCAGAAACACAACATGGAACAA
TAGTTATCAGAGTGCAATATGAAGGGGACGGCTCTCCATGCAAGATCCCTTTTGAGATAATGGATTTGGAAAAAAGA
CATGTCTTAGGTCGCCTGATTACAGTCAACCCAATTGTGACAGAAAAAGATAGCCCAGTCAACATAGAAGCAGAACC
TCCATTTGGAGACAGCTACATCATCATAGGAGTAGAGCCGGGACAACTGAAGCTCAACTGGTTTAAGAAAGGAAGTT
CTATCGGCCAAATGTTTGAGACAACAATGAGGGGGGCGAAGAGAATGGCCATTTTAGGTGACACAGCCTGGGATTTT
GGATCCTTGGGAGGAGTGTTTACATCTATAGGAAAGGCTCTCCACCAAGTCTTTGGAGCAATCTATGGAGCTGCCTT
CAGTGGGGTTTCATGGACTATGAAAATCCTCATAGGAGTCATTATCACATGGATAGGAATGAATTCACGCAGCACCT
CACTGTCTGTGACACTAGTATTGGTGGGAATTGTGACACTGTATTTGGGAGTCATGGTGCAGGCC

大写字母 :E 编码序列 ;小写字母 :prM 编码序列

> BID/V585 - prME核苷酸序列 (SEQ ID NO: 5)

ttccatttaaccacacgtaatggagaaccacacatgatcgttggtaggcaagagaaagggaaaagtcttct
gtttaaaacagaggatggtgttaacatgtgcaccctcatggccatagaccttggtgaattgtgtgaagatacaatc
acgtacaagtgccccctcctcaggcaaaatgaaccagaagacatagattgttggtgcaactctacgtccacatgggt
aacttatgggacatgtaccaccacaggagaacacagaagagaaaaaagatcagtggcactcgttccacatgtgggca
tgggactggagacacgaactgaaacatggatgtcatcagaaggggcctggaaacatgttcagagaattgaaacctgg
atcttgagacatccaggctttaccataatggcagcaatcctggcatataccataggaacgacacatttccaaagggc
tctgatcttcattttactgacagccgttgctccttcaatgacaATGCGTTGCATAGGAATATCAAATAGAGACTTCG
TAGAAGGGGTTTCAGGAGGAAGTTGGGTTGACATAGTCTTAGAACATGGAAGTTGTGTGACGACGATGGCAAAAAAT

AAACCAACATTGGATTTTGAACTGATAAAAACAGAAGCCAAACAACCTGCCACTCTAAGGAAGTACTGTATAGAAGC
AAAGCTGACCAATACAACAACAGAATCTCGTTGCCCAACACAAGGGGAACCCAGTCTAAATGAAGAGCAGGACAAAA
GGTTCATCTGCAAACACTCCATGGTAGACAGAGGATGGGGAAATGGATGTGGATTATTTGGAAAGGGAGGCATTGTG
ACCTGTGCTATGTTCACATGCAAAAAGAACATGGAAGGAAAAGTCGTGCAGCCAGAAAATCTGGAATACACCATCGT
GATAACACCTCACTCAGGAGAAGAGCACGCTGTAGGTAATGACACAGGAAAGCATGGCAAGGAAATCAAAATAACAC
CACAGAGCTCCATCACAGAAGCAGAACTGACAGGCTATGGCACTGTCACGATGGAGTGCTCTCCGAGAACGGGCCTC
GACTTCAATGAGATGGTACTGCTGCAGATGGAAGACAAAGCTTGGCTGGTGCACAGGCAATGGTTCCTAGACCTGCC
GTTACCATGGCTACCCGGAGCGGACACACAAGGATCAAATTGGATACAGAAAGAGACGTTGGTCACTTTCAAAAATC
CCCACGCGAAGAAACAGGACGTCGTTGTTTTAGGATCTCAAGAAGGGGCCATGCACACGGCACTTACAGGGGCCACA
GAAATCCAGATGTCATCAGGAAACTTACTGTTCACAGGACATCTCAAGTGTAGGCTGAGAATGGACAAATTACAGCT
TAAAGGAATGTCATACTCTATGTGTACAGGAAAGTTTAAAATTGTGAAGGAAATAGCAGAAACACAACATGGAACAA
TAGTTATCAGAGTACAATATGAAGGGGACGGCTCTCCATGTAAGATTCCTTTTGAGATAATGGATTTGGAAAAAAGA
CACGTCCTAGGTCGCCTGATTACAGTGAACCCAATCGTAACAGAAAAAGATAGCCCAGTCAACATAGAAGCAGAACC
TCCATTCGGAGACAGCTACATCATCATAGGAGTAGAGCCGGGACAATTGAAACTCAATTGGTTCAAGAAGGGAAGTT
CCATTGGCCAAATGTTTGAGACAACAATGAGAGGAGCGAAGAGAATGGCCATTTTAGGTGACACAGCCTGGGATTTT
GGATCCCTGGGAGGAGTGTTTACATCTATAGGAAAGGCTCTCCACCAAGTTTTCGGAGCAATCTATGGGGCTGCTTT
TAGTGGGGTCTCATGGACTATGAAAATCCTCATAGGAGTTATTATCACATGGATAGGAATGAATTCACGTAGCACCT
CACTGTCTGTGTCACTAGTATTGGTGGGAGTCGTGACACTGTACTTGGGGGTTATGGTGCAGGCT

>PR/DB023 prME核苷酸序列(SEQ ID NO: 6)

ttccatttaaccacacgtaatggagaaccacacatgatcgttggtaggcaagagaaagggaaaagtcttct
gttcaaaacagaggatggtgttaacatgtgtaccctcatggccatagaccttggtgaattgtgtgaagatacaatc
acgtacaagtgcccccctcctcaggcaaaatgaaccagaagacatagattgttggtgcaactctacgtccacatgggt
aacttatgggacatgtaccaccacaggagaacacagaagagaaaaaagatcagtggcactcgttccacatgtgggca
tgggactggagacacgaactgaaacatggatgtcatcagaaggggcctggaaacatgttcagagaattgaaacctgg
atattgagacatccaggctttaccataatggcagcaatcctggcatataccataggaacgacacatttccaaagggc
tctgatcttcattttactgacagccgtcgctccttcaatgacaATGCGTTGCATAGGAATATCAAATAGAGACTTCG
TAGAAGGGGTTTCAGGAGGAAGTTGGGTTGACATAGTCTTAGAACATGGAAGTTGTGTGACGACGATGGCAAAAAAT
AAACCAACATTGGATTTTGAACTGATAAAAACAGAAGCCAAACAACCTGCCACTCTAAGGAAGTACTGTATAGAAGC
AAAGCTGACCAATACAACAACAGAATCTCGTTGCCCAACACAAGGGGAACCCAGTCTAAATGAAGAGCAGGACAAAA
GGTTCATCTGCAAACACTCCATGGTAGACAGAGGATGGGGAAATGGATGTGGATTATTTGGAAAAGGAGGCATTGTA
ACCTGTGCTATGTTCACATGCAAAAAGAACATGGAAGGAAAAGTTGTGCTGCCAGAAAATCTGGAATACACCATCGT
GATAACACCTCACTCAGGAGAAGAGCACGCTGTAGGTAATGACACAGGAAAACATGGCAAGGAAATTAAAATAACAC
CACAGAGTTCCATCACAGAAGCAGAACTGACAGGCTATGGCACTGTCACGATGGAGTGCTCTCCGAGAACGGGCCTC
GACTTCAATGAGATGGTGCTGCTGCAGATGGAAGACAAAGCCTGGCTGGTGCACAGGCAATGGTTCCTAGATCTGCC
GTTACCATGGCTACCCGGAGCGGACACACAAGGATCAAATTGGATACAGAAAGAGACGTTGGTCACTTTCAAAAATC
CCCACGCGAAGAAACAGGACGTCGTTGTTTTAGGATCTCAAGAAGGGGCCATGCACACGGCACTTACAGGGGCCACA
GAAATCCAGATGTCATCAGGAAACTTACTGTTCACAGGACATCTCAAGTGTAGGCTGAGAATGGACAAATTACAGCT
TAAAGGAATGTCATACTCTATGTGTACAGGAAAGTTTAAAATTGTGAAGGAAATAGCAGAAACACAACATGGAACAA
TAGTTATCAGAGTACAATATGAAGGGGACGGCTCTCCATGTAAGATTCCTTTTGAGATAATGGATTTAGAAAAAAGA

CACGTCCTAGGTCGCCTGATTACAGTGAACCCAATCGTAACAGAAAAAGATAGCCCAGTCAACATAGAAGCAGAACC
TCCATTCGGAGACAGCTACATCATCATAGGAGTAGAGCCGGGACAATTGAAACTCAATTGGTTCAAGAAGGGAAGTT
CCATTGGCCAAATGTTTGAGACAACAATGAGAGGAGCGAAGAGAATGGCCATTTTAGGTGACACAGCCTGGGATTTT
GGATCCCTGGGAGGAGTGTTTACATCTATAGGAAAGGCTCTCCACCAAGTTTTCGGAGCAATCTATGGGGCTGCTTT
TAGTGGGGTCTCATGGACTATGAAAATCCTCATAGGAGTTATCATCACATGGATAGGAATGAATTCACGTAGCACCT
CACTGTCTGTGTCACTAGTATTGGTGGGAGTCGTGACACTGTACTTGGGGGTTATGGTGCAGGCT

>MD1280 prME核苷酸序列(SEQ ID NO: 7)

ttccatttaaccacacgaaatggagaaccacacatgatcgttggcagacaagagaaagggaaaagccttct
gtttaaaacagaggatggtgtgaacatgtgtaccctcatggccattgatcttggtgaattgtgtgaagatacaatc
acgtacaagtgccccctcctcaggcagaatgaaccagaagatatagattgttggtgcaactccacgtccacatgggt
aacttatgggacgtgtaccaccacaggagaacacagaagagaaaaaagatcagtggcactcgttccacatgtgggta
tgggactggagacacgaactgaaacatggatgtcgtcagaaggggcctggaaacacgctcagagaattgaaacttgg
atcttgagacatccaggctttaccataatggcagcaatcctggcatataccgtaggaacgacacatttccaaagggc
cctgattttcatcttactggcagctgtcgctccttcaatgacaATGCGTTGCATAGGAATATCAAATAGAGACTTTG
TAGAAGGGGTTTCAGGAGGAAGCTGGGTTGACATAGTCTTAGAACATGGAAGTTGTGTGACGACAATGGCAAAAAAT
AAACCAACACTGGATTTTGAACTGATAAAAACAGAAGCCAAACAACCTGCCACTCTAAGGAAGTACTGTATAGAGGC
AAAGCTGACCAATACAACAACAGAATCTCGTTGCCCAACACAAGGGGAACCCAGTCTAAATGAAGAGCAGGACAAAA
GGTTCGTCTGCAAACACTCCATGGTAGACAGAGGATGGGGAAATGGATGTGGATTATTTGGAAAGGGAGGCATTGTG
ACCTGTGCTATGTTCACATGCAAAAAGAACATGGAAGGAAAAATCGTGCAACCAGAAAATTTGGAATACACCATCGT
GATAACACCTCACTCAGGAGAAGAGCACGCTGTAGGTAATGACACAGGAAAACATGGTAAGGAAATTAAAATAACAC
CACAGAGTTCCATCACAGAAGCAGAACTGACAGGCTATGGCACAGTCACGATGGAGTGCTCTCCGAGAACGGGCCTT
GACTTCAATGAGATGGTGCTGCTGCAGATGGAAGATAAAGCTTGGCTGGTGCACAGGCAATGGTTCCTAGACCTGCC
GTTACCATGGCTACCCGGAGCGGACACACAAGGATCAAATTGGATACAGAAAGAGACATTGGTCACTTTCAAAAATC
CCCACGCGAAGAAGCAGGATGTCGTTGTTTTAGGATCTCAAGAAGGAGCCATGCACACGGCACTCACAGGGGCCACA
GAAATCCAGATGTCATCAGGAAACTTACTATTCACAGGACATCTCAAATGCAGGCTGAGAATGGACAAACTACAGCT
CAAAGGAATGTCATACTCTATGTGTACAGGAAAGTTTAAAATTGTGAAGGAAATAGCAGAAACACAACATGGAACAA
TAGTTATCAGAGTACAATATGAAGGAGACGGCTCTCCATGTAAGATCCCTTTTGAAATAATGGATTTGGAAAAAAGA
CATGTCTTAGGTCGCCTGATTACAGTTAATCCGATCGTAACAGAAAAAGATAGCCCAGTCAACATAGAAGCAGAACC
TCCATTCGGAGACAGCTACATCATTATAGGAGTAGAGCCGGGACAATTGAAACTCAACTGGTTCAAGAAAGGAAGTT
CCATCGGCCAAATGTTTGAGACGACAATGAGAGGAGCAAAGAGAATGGCCATTTTAGGTGACACAGCCTGGGATTTT
GGATCTCTGGGAGGAGTGTTTACATCTATAGGAAAGGCTCTCCACCAAGTTTTCGGAGCAATCTATGGGGCTGCCTT
TAGTGGGGTTTCATGGACTATGAAAATCCTCATAGGAGTCATCATCACATGGATAGGAATGAATTCACGTAGCACCT
CACTGTCTGTGTCACTAGTATTGGTGGGAATCATAACACTGTACTTGGGAGCTATGGTGCAGGCT

四个所选毒株相应的蛋白质prM-E序列

>LAV2 prME蛋白序列(SEQ ID NO: 8)

fhlttrngephmivsrqekgksllfktevgvnmctlmamdlgelcedtitykcpllrqnepedidcwcnst
stwvtygtcttmgehrrekrsvalvphvgmgletrtetwmssegawkhvqrietwilrhpgftmmaailaytigtt
hfqralifilltavtpsmtMRCIGMSNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRK
YCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFRCKKNMEGKVVQPENL

EYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMENKAWLVHRQW
FLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRM
DKLQLKGMSYSMCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAI
YGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVTLVLVGIVTLYLGVMVQA

>LAV2 E蛋白序列 (SEQ ID NO: 13)

MRCIGMSNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTE
SRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFRCKKNMEGKVVQPENLEYTIVITPHSGE
EHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGA
DTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSM
CTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYI
IIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTM
KILIGVIITWIGMNSRSTSLSVTLVLVGIVTLYLGVMVQA

>LAV2 M蛋白序列 (SEQ ID NO: 19)

svalvphvgmgletrtetwmssegawkhvqrietwilrhpgftmmaailaytigtthfqralifilltavtps
mt

>BID/V585 prME蛋白序列 (SEQ ID NO: 9)

fhlttrngephmivgrqekgksllfktedgvnmctlmaidlgelcedtitykcpllrqnepedidcwcnst
stwvtygtctttgehrrekrsvalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtt
hfqralifilltavapsmtMRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRK
YCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKVVQPENL
EYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQW
FLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRM
DKLQLKGMSYSMCTGKFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAI
YGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA

>BID/V585 E蛋白序列 (SEQ ID NO: 14)

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTE
SRCPTQGEPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKVVQPENLEYTIVITPHSGE
EHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGA
DTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSM
CTGKFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYI
IIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTM
KILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA

>BID/V585 M蛋白序列 (SEQ ID NO: 20)

svalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtthfqralifilltavaps
mt

>PR/DB023 prME蛋白序列 (SEQ ID NO: 10)

fhlttrngephmivgrqekgksllfktedgvnmctlmaidlgelcedtitykcpllrqnepedidcwcnst

stwvtygtctttgehrrekrsvalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtt
hfqralifilltavapsmtMRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRK
YCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKVVLPENL
EYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQW
FLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRM
DKLQLKGMSYSMCTGKFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAI
YGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA

>PR/DB023 E蛋白序列(SEQ ID NO: 15)

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTE
SRCPTQGEPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKVVLPENLEYTIVITPHSGE
EHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGA
DTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSM
CTGKFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYI
IIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTM
KILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA

>PR/DB023 M蛋白序列(SEQ ID NO: 21)

svalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtthfqralifilltavaps
mt

>MD1280 prME蛋白序列(SEQ ID NO: 11)

fhlttrngephmivgrqekgksllfktedgvnmctlmaidlgelcedtitykcpllrqnepedidcwcnst
stwvtygtctttgehrrekrsvalvphvgmgletrtetwmssegawkhaqrietwilrhpgftimaailaytvgtt
hfqralifillaavapsmtMRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRK
YCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENL
EYTIVITPHSGEEHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQW
FLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRM
DKLQLKGMSYSMCTGKFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVN
IEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAI
YGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGIITLYLGAMVQA

>MD1280 E蛋白序列(SEQ ID NO: 16)

MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTE
SRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENLEYTIVITPHSGE
EHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGA
DTQGSNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSM
CTGKFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYI
IIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTM
KILIGVIITWIGMNSRSTSLSVSLVLVGIITLYLGAMVQA

>MD1280 M蛋白序列(SEQ ID NO: 22)

svalvphvgmgletrtetwmssegawkhaqrietwilrhpgftimaailaytvgtthfqralifillaavaps

mt

>共有 M序列 (SEQ ID NO: 17)

svalvphvgmgletrtetwmssegawkhvqrietwilrhpgftimaailaytigtthfqralifilltavaps

mt

实施例 3:相当于优化血清型 2嵌合病毒的 cDNA克隆的构建和编码病毒的产生

基本按照 Chambers 等人 (1999, J. Virology 73(4): 3095-3101) 的教导,采用 Chimerivax™技术来实现相当于优化血清型 2 毒株的嵌合登革热病毒的构建。还可参照国际专利申请 WO 98/37911、WO 03/101397、WO 07/021672、WO 08/007021、WO 08/047023 和 WO 08/065315,其详细描述了用于构建 CYD-1、CYD2、CYD-3 和 CYD-4 的类似方法。然而简单地说,如下构建相当于优化血清型 2 毒株的嵌合登革热病毒 (注意 :优化嵌合登革热病毒使用 YF 毒株 YF17D204 (YF-VAX(R), Sanofi-Pasteur, Swiftwater, PA, USA) 的基因组骨架构建)。

[0223] 质粒 pSP1101的构建

YF-VAX cDNA 克隆 -pJSY2284.1 (pACYC YF-Vax 5-3) 的构建

构建了 YF-VAX 的全长感染性 cDNA 克隆。全长感染性 cDNA 克隆以 YF-VAX 的序列为基础。低拷贝数质粒 pACYC177 (New England Biolabs, Inc., Ipswich, MA, USA) 用来装配全长 cDNA 克隆。

[0224] 通过 GeneArt® 合成称为 SP6 YF-Vax 5-3 的 DNA 序列。以利于全长 YF-Vax cDNA 克隆的容易装配的方式设计 SP6 YF-Vax 5-3 的序列。序列长 2897 bp,包含 Xma I-SP6 启动子 ;YF-Vax 5'UTR ;衣壳 ;prM ;M ;E 的部分,其延伸至 Apa I 位点接着用于装配的独特位点 Mlu I-Sap I-Ngo MI-Aat II-Cla I ;NS5 的部分 ;并进一步延伸至 3'UTR 接着 Nru I 位点,其用于脱去。这种合成的 DNA 序列的侧翼是 EcoR V 和 Xho I 位点。在用 EcoR V/Xho I 消化后,然后将该 DNA 片段克隆至低拷贝数质粒 pACYC177 的 Aat II/Xho I 位点以置换 1615bp Aat II/Xho I 片段。所得质粒 pJSY2284.1 (pACYC YF-Vax 5-3) 通过序列分析证实。

[0225] *跨越位点 Apa I、Mlu I、Sap I、Ngo MI、Aat II和 Cla I的 YF-Vax cDNA片段的 RT-PCR和克隆和 YF-vax的全长感染性 cDNA克隆的装配 (pJSY2374.5)*

使黄热病疫苗 YF-VAX 在 Vero 细胞中生长,并浓缩病毒颗粒。从浓缩的病毒中提取 YF-VAX 的病毒 RNA,并通过反转录酶产生 cDNA 拷贝。本文所示 5 个 cDNA 片段经 PCR 扩增、TOPO 克隆、测序,并与 YF-VAX 2003 的序列比较。各片段中发现的 PCR 错误通过位点定向诱变或片段交换纠正。在 TOPO 克隆后有太多序列不同存在于 Ngo MI-Aat II 片段中,因此,该片段通过 GeneArt® 合成。在最终的序列证实后,分离 5 个 DNA 片段 ;Apa I-Mlu I、Mlu I-Sap I、Sap I-Ngo M1、Ngo MI-Aat II 和 Aat II-Cla I,并逐步克隆至质粒 pJSY2284.1 中的独特位点 Apa I、Mlu I、Sap I、Ngo MI、Aat II 和 Cla I 中以得到质粒 pJSY2374.5,其被证实含有 YF-VAX 全长 cDNA 的正确序列。

[0226] 用于来源于 LAV2 毒株的优化嵌合登革热病毒的 cDNA 的构建 (pSP1101)

策略是采用 Chimerivax™技术,在含有 YF-VAX 基因组的 pJSY2374.5 质粒中用 LAV2 毒株的 prM 和 E 基因置换 YF-VAX® 疫苗毒株的 prM 和 E 基因,如前进行以构建 CYD-1、CYD-2、CYD-3 和 CYD-4 登革热疫苗。所得质粒为 pSP1101。

[0227] 在 pJSY2374 中,用于克隆的限制位点为 Xma I 和 Mlu I。这些位点位于 3000 bp 片段的上游和下游,所述片段含有 :SP6 启动子、YF17D 5’UTR、YF17D- 衣壳、YF17D-prM、YF17D-E 和 YF17D-NS1 的 N 末端。相当于该片段但取而代之含有侧翼是 Xma I 和 Mlu I 位点的 LAV2 的 prM 和 E 基因的序列通过 GeneArt® 合成,并克隆至质粒 pMK-RQ (GeneArt®, Life Technologies Ltd, Paisley, U.K.) 中以产生质粒 pMK-RQ-Seq1。质粒 pJSY2374.5 和 pMK-RQ-Seq1 用 Xma I 和 Mlu 1 消化。然后将来自 pMK-RQ-Seq1 的 Xma I-Mlu I 片段插入质粒 pJSY2374.5 中以形成质粒 pSP1101。XL-10 Gold Ultracompetent 细菌 (Agilent Technologies, CA, USA) 用于转化,因为它们适于大的质粒。在第二步中,将阳性克隆转移至 One Shot® TOP10 大肠杆菌 (*E. coli*) (Life Technologies Ltd,Paisley,U.K.) 中,这允许大量扩增大尺寸质粒。

[0228] 质粒 pSP1101 因此允许用 YF-VAX 复制引擎表达 LAV2 毒株 prM 和 E 蛋白。所得嵌合病毒命名为 CYD-LAV。测序分析显示与原始序列相比无突变。

[0229] *用于毒株 BID-V585、PR/DB023和 MD1280的相应质粒的构建*

采用类似于上文所述的策略以构建对应于血清型 2 毒株 BID-V585、PR/DB023 和 MD1280 的质粒。这些质粒命名为 pSP1102 (BID-V585)、pSP1103 (PR/DB023) 和 pSP1104 (MD1280)。从这些质粒产生的所得嵌合病毒命名为 CYD-BID、CYD-PR 和 CYD-MD。所产生的质粒的序列分析显示与起始序列相比无突变。

[0230] *自质粒 pSP1101、pSP1102、pSP1103和 pSP1104产生嵌合病毒*

RNA 的体外转录和病毒的产生按之前所描述的进行 (Guirakhoo F 等 J. Virol. 2001 ; 75:7290-304)。

[0231] 实施例 4:在猴模型中评价优化血清型 2嵌合病毒的免疫原性和病毒血症

在猴中评价免疫原性和病毒血症

研究设计

确定了各含 4 只食蟹猴 (Cynomolgus monkey) 的 4 个组。4 组接受下列制剂 (含有 5 log_{10} $CCID_{50}$的各 CYD 登革热血清型) :

1. 对照四价制剂,即包含 CYD-1、CYD-2、CYD-3 和 CYD-4 的制剂。

[0232] 2. CYD-LAV 四价制剂,即包含 CYD-1、CYD-3、CYD-4 和 CYD-LAV 的制剂。

[0233] 3. CYD-MD 四价制剂,即包含 CYD-1、CYD-3、CYD-4 和 CYD-MD 的制剂。

[0234] 4. CYD-PR 四价制剂,即包含 CYD-1、CYD-3、CYD-4 和 CYD-PR 的制剂。

[0235] 如前所述,猴相隔 2 个月接受 2 剂 (Guy B 等, Am J Trop Med Hyg. 2009 ; 80(2):302-11)。

[0236] 结果

按 Guy B., 等, Am. J. Trop. Med. Hyg. 2009 ;80(2): 302-11 中描述的材料与方法,测定免疫原性 (SN_{50}中和反应) 和病毒血症。

[0237] 表 6 :用优化的嵌合登革热血清型 2 病毒免疫的猴中的 SN_{50}中和反应

		PD1				PD2			
		DEN1	DEN2	DEN3	DEN4	DEN1	DEN2	DEN3	DEN4
对照 CYD TV	应答者	4/4	2/4	1/4	4/4	4/4	1/4	4/4	4/4
	GMT	**27**	**5**	**7**	**636**	**71**	**8**	**35**	**425**
CYD-LAV TV	应答者	4/4	2/4	4/4	4/4	4/4	4/4	4/4	4/4
	GMT	**95**	**63**	**19**	**477**	**189**	**95**	**80**	**477**
CYD-MD TV	应答者	4/4	4/4	3/4	4/4	4/4	4/4	4/4	4/4
	GMT	**33**	**100**	**8**	**63**	**35**	**63**	**16**	**100**
CYD-PR TV	应答者	3/4	4/4	1/4	4/4	4/4	4/4	4/4	4/4
	GMT	**27**	**63**	**7**	**168**	**109**	**84**	**38**	**212**

PD :给药后 ;TV :四价制剂

无论给予的血清型 2 嵌合病毒如何,未观察到血清型 2 病毒血症。有关针对 DEN2 的免疫原性反应,与对照制剂相比,包含 CYD-LAV、CYD-MD 和 CYD-PR 的四价制剂表明较高的反应(GMT 和响应动物的数目两者)(参见表 6)。

[0238] 实施例 5. 在墨西哥的黄病毒 -幼稚成人中评价四价登革热疫苗制剂

本研究的目的是对包含 CYD-1 (即从 DEN1 PUO359 (TYP 1 140) 的 prM 和 E 序列产生的特殊的 Chimerivax 登革热血清型 1 (CYD-1) 毒株)、VDV2、CYD-3 (即从 DEN3 PaH881/88 的 prM 和 E 序列产生的特殊的 Chimerivax 登革热血清型 3 (CYD-3) 毒株) 和 CYD-4 (即从 DEN4 1228 (TVP 980) 的 prM 和 E 序列产生的特殊的 Chimerivax 登革热血清型 4 (CYD-4) 毒株) 的混合四价登革热疫苗的免疫原性和病毒血症与包含 CYD-1、CYD-2 (即自 DEN2 PUO218 的 prM 和 E 序列产生的特殊的 Chimerivax 登革热血清型 2 (CYD-2) 毒株)、CYD-3 和 CYD-4 的四价登革热疫苗的免疫原性和病毒血症进行比较。有关用于本研究的特定 CYD-1、CYD-2、CYD-3 和 CYD-4 的更多详情参见实施例 1。

[0239] VDV2 毒株的相关核苷酸和蛋白质序列如下 :

>VDV2核苷酸序列 (SEQ ID NO: 24)

AGUUGUUAGUCUACGUGGACCGACAAAGACAGAUUCUUUGAGGGAGCUAAGCUCAAUGUAGUUCUAACAGUUU
UUUAAUUAGAGAGCAGAUCUCUGAUGAAUAACCAACGGAAAAAGGCGAAAAACACGCCUUUCAAUAUGCUGAAACGC
GAGAGAAACCGCGUGUCGACUGUGCAACAGCUGACAAAGAGAUUCUCACUUGGAAUGCUGCAGGGACGAGGACCAUU
AAAACUGUUCAUGGCCCUGGUGGCGUUCCUUCGUUUCCUAACAAUCCCACCAACAGCAGGGAUAUUGAAGAGAUGGG
GAACAAUUAAAAAAUCAAAAGCUAUUAAUGUUUUGAGAGGGUUCAGGAAAGAGAUUGGAAGGAUGCUGAACAUCUUG
AAUAGGAGACGCAGAUCUGCAGGCAUGAUCAUUAUGCUGAUUCCAACAGUGAUGGCGUUCCAUUUAACCACACGUAA
CGGAGAACCACACAUGAUCGUCAGCAGACAAGAGAAAGGGAAAAGUCUUCUGUUUAAAACAGAGGUUGGCGUGAACA
UGUGUACCCUCAUGGCCAUGGACCUUGGUGAAUUGUGUGAAGACACAAUCACGUACAAGUGUCCCCUUCUCAGGCAG
AAUGAGCCAGAAGACAUAGACUGUUGGUGCAACUCUACGUCCACGUGGGUAACUUAUGGGACGUGUACCACCAUGGG

AGAACAUAGAAGAGAAAAAAGAUCAGUGGCACUCGUUCCACAUGUGCGAAUGGGACUGGAGACACGAACUGAAACAU
GGAUGUCAUCAGAAGGGGCCUGGAAACAUGUCCAGAGAAUUGAAACUUGGAUCUUGAGACAUCCAGGCUUCACCAUG
AUGGCAGCAAUCCUGGCAUACACCAUAGGAACGACACAUUUCCAAAGAGCCCUGAUUUUCAUCUUACUGACAGCUGU
CACUCCUUCAAUGACAAUGCGUUGCAUAGGAAUGUCAAAUAGAGACUUUGUGGAAGGGGUUUCAGGAGGAAGCUGGG
UUGACAUAGUCUUAGAACAUGGAAGCUGUGUGACGACGAUGGCAAAAAACAAACCAACAUUGGAUUUUGAACUGAUA
AAAACAGAAGCCAAACAGCCUGCCACCCUAAGGAAGUACUGUAUAGAGGCAAAGCUAACCAACACAACAACAGAAUC
UCGCUGCCCAACACAAGGGGAACCCAGCCUAAAUGAAGAGCAGGACAAAAGGUUCGUCUGCAAACACUCCAUGGUAG
ACAGAGGAUGGGGAAAUGGAUGUGGACUAUUUGGAAAGGGAGGCAUUGUGACCUGUGCUAUGUUCAGAUGCAAAAAG
AACAUGGAAGGAAAAGUUGUGCAACCAGAAAACUUGGAAUACACCAUUGUGAUAACACCUCACUCAGGGGAAGAGCA
UGCAGUCGGAAAUGACACAGGAAAACAUGGCAAGGAAAUCAAAAUAACACCACAGAGUUCCAUCACAGAAGCAGAAU
UGACAGGUUAUGGCACUGUCACAAUGGAGUGCUCUCCAAGAACGGGCCUCGACUUCAAUGAGAUGGUGUUGCUGCAG
AUGGAAAAUAAAGCUUGGCUGGUGCACAGGCAAUGGUUCCUAGACCUGCCGUUACCAUGGUUGCCCGGAGCGGACAC
ACAAGAGUCAAAUUGGAUACAGAAGGAGACAUUGGUCACUUUCAAAAAUCCCCAUGCGAAGAAACAGGAUGUUGUUG
UUUUAGGAUCCCAAGAAGGGGCCAUGCACACAGCACUUACAGGGGCCACAGAAAUCCAAAUGUCAUCAGGAAACUUA
CUCUUCACAGGACAUCUCAAGUGCAGGCUGAGAAUGGACAAGCUACAGCUCAAAGGAAUGUCAUACUCUAUGUGCAC
AGGAAAGUUUAAAGUUGUGAAGGAAAUAGCAGAAACACAACAUGGAACAAUAGUUAUCAGAGUGCAAUAUGAAGGGG
ACGGCUCUCCAUGCAAGAUCCCUUUUGAGAUAAUGGAUUUGGAAAAAAGACAUGUCUUAGGUCGCCUGAUUACAGUC
AACCCAAUUGUGACAGAAAAAGAUAGCCCAGUCAACAUAGAAGCAGAACCUCCAUUUGGAGACAGCUACAUCAUCAU
AGGAGUAGAGCCGGGACAACUGAAGCUCAACUGGUUUAAGAAAGGAAGUUCUAUCGGCCAAAUGUUUGAGACAACAA
UGAGGGGGGCGAAGAGAAUGGCCAUUUUAGGUGACACAGCCUGGGAUUUUGGAUCCUUGGGAGGAGUGUUUACAUCU
AUAGGAAAGGCUCUCCACCAAGUCUUUGGAGCAAUCUAUGGAGCUGCCUUCAGUGGGGUUUCAUGGACUAUGAAAAU
CCUCAUAGGAGUCAUUAUCACAUGGAUAGGAAUGAAUUCACGCAGCACCUCACUGUCUGUGACACUAGUAUUGGUGG
GAAUUGUGACACUGUAUUUGGGAGUCAUGGUGCAGGCCGAUAGUGGUUGCGUUGUGAGCUGGAAAAACAAAGAACUG
AAAUGUGGCAGUGGGAUUUUCAUCACAGACAACGUGCACACAUGGACAGAACAAUACAAAUUCCAACCAGAAUCCCC
UUCAAAACUAGCUUCAGCUAUCCAGAAAGCCCAUGAAGAGGACAUUUGUGGAAUCCGCUCAGUAACAAGACUGGAGA
AUCUGAUGUGGAAACAAAUAACACCAGAAUUGAAUCACAUUCUAUCAGAAAAUGAGGUGAAGUUAACUAUUAUGACA
GGAGACAUCAAAGGAAUCAUGCAGGCAGGAAAACGAUCUCUGCGGCCUCAGCCCACUGAGCUGAAGUAUUCAUGGAA
AACAUGGGGCAAAGCAAAAAUGCUCUCUACAGAGUCUCAUAACCAGACCUUUCUCAUUGAUGGCCCCGAAACAGCAG
AAUGCCCCAACACAAAUAGAGCUUGGAAUUCGUUGGAAGUUGAAGACUAUGGCUUUGGAGUAUUCACCACCAAUAUA
UGGCUAAAAUUGAAAGAAAAACAGGAUGUAUUCUGCGACUCAAAACUCAUGUCAGCGGCCAUAAAAGACAACAGAGC
CGUCCAUGCCGAUAUGGGUUAUUGGAUAGAAAGUGCACUCAAUGACACAUGGAAGAUAGAGAAAGCCUCUUUCAUUG
AAGUUAAAAACUGCCACUGGCCAAAAUCACACACCCUCUGGAGCAAUGGAGUGCUAGAAAGUGAGAUGAUAAUUCCA
AAGAAUCUCGCUGGACCAGUGUCUCAACACAACUAUAGACCAGGCUACCAUACACAAAUAACAGGACCAUGGCAUCU
AGGUAAGCUUGAGAUGGACUUUGAUUUCUGUGAUGGAACAACAGUGGUAGUGACUGAGGACUGCGGAAAUAGAGGAC
CCUCUUUGAGAACAACCACUGCCUCUGGAAAACUCAUAACAGAAUGGUGCUGCCGAUCUUGCACAUUACCACCGCUA
AGAUACAGAGGUGAGGAUGGGUGCUGGUACGGGAUGGAAAUCAGACCAUUGAAGGAGAAAGAAGAGAAUUUGGUCAA
CUCCUUGGUCACAGCUGGACAUGGGCAGGUCGACAACUUUUCACUAGGAGUCUUGGGAAUGGCAUUGUUCCUGGAGG
AAAUGCUUAGGACCCGAGUAGGAACGAAACAUGCAAUACUACUAGUUGCAGUUUCUUUUGUGACAUUGAUCACAGGG
AACAUGUCCUUUAGAGACCUGGGAAGAGUGAUGGUUAUGGUAGGCGCCACUAUGACGGAUGACAUAGGUAUGGGCGU

GACUUAUCUUGCCCUACUAGCAGCCUUCAAAGUCAGACCAACUUUUGCAGCUGGACUACUCUUGAGAAAGCUGACCU
CCAAGGAAUUGAUGAUGACUACUAUAGGAAUUGUACUCCUCUCCCAGAGCACCAUACCAGAGACCAUUCUUGAGUUG
ACUGAUGCGUUAGCCUUAGGCAUGAUGGUCCUCAAAAUGGUGAGAAAUAUGGAAAAGUAUCAAUUGGCAGUGACUAU
CAUGGCUAUCUUGUGCGUCCCAAACGCAGUGAUAUUACAAAACGCAUGGAAAGUGAGUUGCACAAUAUUGGCAGUGG
UGUCCGUUUCCCCACUGUUCUUAACAUCCUCACAGCAAAAAACAGAUUGGAUACCAUUAGCAUUGACGAUCAAAGGU
CUCAAUCCAACAGCUAUUUUUCUAACAACCCUCUCAAGAACCAGCAAGAAAAGGAGCUGGCCAUUAAAUGAGGCUAU
CAUGGCAGUCGGGAUGGUGAGCAUUUUAGCCAGUUCUCUCCUAAAAAAUGAUAUUCCCAUGACAGGACCAUUAGUGG
CUGGAGGGCUCCUCACUGUGUGCUACGUGCUCACUGGACGAUCGGCCGAUUUGGAACUGGAGAGAGCAGCCGAUGUC
AAAUGGGAAGACCAGGCAGAGAUAUCAGGAAGCAGUCCAAUCCUGUCAAUAACAAUAUCAGAAGAUGGUAGCAUGUC
GAUAAAAAAUGAAGAGGAAGAACAAACACUGACCAUACUCAUUAGAACAGGAUUGCUGGUGAUCUCAGGACUUUUUC
CUGUAUCAAUACCAAUCACGGCAGCAGCAUGGUACCUGUGGGAAGUGAAGAAACAACGGGCCGGAGUAUUGUGGGAU
GUUCCUUCACCCCCACCCAUGGGAAAGGCUGAACUGGAAGAUGGAGCCUAUAGAAUUAAGCAAAAAGGGAUUCUUGG
AUAUUCCCAGAUCGGAGCCGGAGUUUACAAAGAAGGAACAUUCCAUACAAUGUGGCAUGUCACACGUGGCGCUGUUC
UAAUGCAUAAAGGAAAGAGGAUUGAACCAACAUGGGCGGACGUCAAGAAAGACCUAAUAUCAUAUGGAGGAGGCUGG
AAGUUAGAAGGAGAAUGGAAGGAAGGAGAAGAAGUCCAGGUAUUGGCACUGGAGCCUGGAAAAAAUCCAAGAGCCGU
CCAAACGAAACCUGGUCUUUUCAAAACCAACGCCGGAACAAUAGGUGCUGUAUCUCUGGACUUUUCUCCUGGAACGU
CAGGAUCUCCAAUUAUCGACAAAAAAGGAAAAGUUGUGGGUCUUUAUGGUAAUGGUGUUGUUACAAGGAGUGGAGCA
UAUGUGAGUGCUAUAGCCCAGACUGAAAAAAGCAUUGAAGACAACCCAGAGAUCGAAGAUCACAUUUUCCGAAAGAG
AAGACUGACCAUCAUGGACCUCCACCCAGGAGCGGGAAAGACGAAGAGAUACCUUCCGGCCAUAGUCAGAGAAGCUA
UAAAACGGGGUUUGAGAACAUUAAUCUUGGCCCCCACUAGAGUUGUGGCAGCUGAAAUGGAGGAAGCCCUUAGAGGA
CUUCCAAUAAGAUACCAGACCCCAGCCAUCAGAGCUGAGCACACCGGGCGGGAGAUUGUGGACCUAAUGUGUCAUGC
CACAUUUACCAUGAGGCUGCUAUCACCAGUUAGAGUGCCAAACUACAACCUGAUUAUCAUGGACGAAGCCCAUUUCA
CAGACCCAGCAAGUAUAGCAGCUAGAGGAUACAUCUCAACUCGAGUGGAGAUGGGUGAGGCAGCUGGGAUUUUUAUG
ACAGCCACUCCCCCGGGAAGCAGAGACCCAUUUCCUCAGAGCAAUGCACCAAUCAUAGAUGAAGAAAGAGAAAUCCC
UGAACGCUCGUGGAAUUCCGGACAUGAAUGGGUCACGGAUUUUAAAGGGAAGACUGUUUGGUUCGUUCCAAGUAUAA
AAGCAGGAAAUGAUAUAGCAGCUUGCCUGAGGAAAAAUGGAAAGAAAGUGAUACAACUCAGUAGGAAGACCUUUGAU
UCUGAGUAUGUCAAGACUAGAACCAAUGAUUGGGACUUCGUGGUUACAACUGACAUUUCAGAAAUGGGUGCCAAUUU
CAAGGCUGAGAGGGUUAUAGACCCCAGACGCUGCAUGAAACCAGUCAUACUAACAGAUGGUGAAGAGCGGGUGAUUC
UGGCAGGACCUAUGCCAGUGACCCACUCUAGUGCAGCACAAAGAAGAGGGAGAAUAGGAAGAAAUCCAAAAAAUGAG
AAUGACCAGUACAUAUACAUGGGGGAACCUCUGGAAAAUGAUGAAGACUGUGCACACUGGAAAGAAGCUAAAAUGCU
CCUAGAUAACAUCAACACGCCAGAAGGAAUCAUUCCUAGCAUGUUCGAACCAGAGCGUGAAAAGGUGGAUGCCAUUG
AUGGCGAAUACCGCUUGAGAGGAGAAGCAAGGAAAACCUUUGUAGACUUAAUGAGAAGAGGAGACCUACCAGUCUGG
UUGGCCUACAGAGUGGCAGCUGAAGGCAUCAACUACGCAGACAGAAGGUGGUGUUUUGAUGGAGUCAAGAACAACCA
AAUCCUAGAAGAAAACGUGGAAGUUGAAAUCUGGACAAAAGAAGGGGAAAGGAAGAAAUUGAAACCCAGAUGGUUGG
AUGCUAGGAUCUAUUCUGACCCACUGGCGCUAAAAGAAUUUAAGGAAUUUGCAGCCGGAAGAAAGUCUCUGACCCUG
AACCUAAUCACAGAAAUGGGUAGGCUCCCAACCUUCAUGACUCAGAAGGCAAGAGACGCACUGGACAACUUAGCAGU
GCUGCACACGGCUGAGGCAGGUGGAAGGGCGUACAACCAUGCUCUCAGUGAACUGCCGGAGACCCUGGAGACAUUGC
UUUUACUGACACUUCUGGCUACAGUCACGGGAGGGAUCUUUUUAUUCUUGAUGAGCGCAAGGGGCAUAGGGAAGAUG
ACCCUGGGAAUGUGCUGCAUAAUCACGGCUAGCAUCCUCCUAUGGUACGCACAAAUACAGCCACACUGGAUAGCAGC

UUCAAUAAUACUGGAGUUUUUUCUCAUAGUUUUGCUUAUUCCAGAACCUGAAAAACAGAGAACACCCCAAGACAACC
AACUGACCUACGUUGUCAUAGCCAUCCUCACAGUGGUGGCCGCAACCAUGGCAAACGAGAUGGGUUUCCUAGAAAAA
ACGAAGAAAGAUCUCGGAUUGGGAAGCAUUGCAACCCAGCAACCCGAGAGCAACAUCCUGGACAUAGAUCUACGUCC
UGCAUCAGCAUGGACGCUGUAUGCCGUGGCCACAACAUUUGUUACACCAAUGUUGAGACAUAGCAUUGAAAAUUCCU
CAGUGAAUGUGUCCCUAACAGCUAUAGCCAACCAAGCCACAGUGUUAAUGGGUCUCGGGAAAGGAUGGCCAUUGUCA
AAGAUGGACAUCGGAGUUCCCCUUCUCGCCAUUGGAUGCUACUCACAAGUCAACCCCAUAACUCUCACAGCAGCUCU
UUUCUUAUUGGUAGCACAUUAUGCCAUCAUAGGGCCAGGACUCCAAGCAAAAGCAACCAGAGAAGCUCAGAAAAGAG
CAGCGGCGGGCAUCAUGAAAAACCCAACUGUCGAUGGAAUAACAGUGAUUGACCUAGAUCCAAUACCUUAUGAUCCA
AAGUUUGAAAAGCAGUUGGGACAAGUAAUGCUCCUAGUCCUCUGCGUGACUCAAGUAUUGAUGAUGAGGACUACAUG
GGCUCUGUGUGAGGCUUUAACCUUAGCUACCGGGCCCAUCUCCACAUUGUGGGAAGGAAAUCCAGGGAGGUUUUGGA
ACACUACCAUUGCGGUGUCAAUGGCUAACAUUUUUAGAGGGAGUUACUUGGCCGGAGCUGGACUUCUCUUUUCUAUU
AUGAAGAACACAACCAACACAAGAAGGGGAACUGGCAACAUAGGAGAGACGCUUGGAGAGAAAUGGAAAAGCCGAUU
GAACGCAUUGGGAAAAAGUGAAUUCCAGAUCUACAAGAAAAGUGGAAUCCAGGAAGUGGAUAGAACCUUAGCAAAAG
AAGGCAUUAAAAGAGGAGAAACGGACCAUCACGCUGUGUCGCGAGGCUCAGCAAAACUGAGAUGGUUCGUUGAGAGA
AACAUGGUCACACCAGAAGGGAAAGUAGUGGACCUCGGUUGUGGCAGAGGAGGCUGGUCAUACUAUUGUGGAGGACU
AAAGAAUGUAAGAGAAGUCAAAGGCCUAACAAAAGGAGGACCAGGACACGAAGAACCCAUCCCCAUGUCAACAUAUG
GGUGGAAUCUAGUGCGUCUUCAAAGUGGAGUUGACGUUUUCUUCAUCCCGCCAGAAAAGUGUGACACAUUAUUGUGU
GACAUAGGGGAGUCAUCACCAAAUCCCACAGUGGAAGCAGGACGAACACUCAGAGUCCUUAACUUAGUAGAAAAUUG
GUUGAACAACAACACUCAAUUUUGCAUAAAGGUUCUCAACCCAUAUAUGCCCUCAGUCAUAGAAAAAAUGGAAGCAC
UACAAAGGAAAUAUGGAGGAGCCUUAGUGAGGAAUCCACUCUCACGAAACUCCACACAUGAGAUGUACUGGGUAUCC
AAUGCUUCCGGGAACAUAGUGUCAUCAGUGAACAUGAUUUCAAGGAUGUUGAUCAACAGAUUUACAAUGAGAUACAA
GAAAGCCACUUACGAGCCGGAUGUUGACCUCGGAAGCGGAACCCGUAACAUCGGGAUUGAAAGUGAGAUACCAAACC
UAGAUAUAAUUGGGAAAAGAAUAGAAAAAAUAAAGCAAGAGCAUGAAACAUCAUGGCACUAUGACCAAGACCACCCA
UACAAAACGUGGGCAUACCAUGGUAGCUAUGAAACAAAACAGACUGGAUCAGCAUCAUCCAUGGUCAACGGAGUGGU
CAGGCUGCUGACAAAACCUUGGGACGUUGUCCCCAUGGUGACACAGAUGGCAAUGACAGACACGACUCCAUUUGGAC
AACAGCGCGUUUUUAAAGAGAAAGUGGACACGAGAACCCAAGAACCGAAAGAAGGCACGAAGAAACUAAUGAAAAUA
ACAGCAGAGUGGCUUUGGAAAGAAUUAGGGAAGAAAAAGACACCCAGGAUGUGCACCAGAGAAGAAUUCACAAGAAA
GGUGAGAAGCAAUGCAGCCUUGGGGGCCAUAUUCACUGAUGAGAACAAGUGGAAGUCGGCACGUGAGGCUGUUGAAG
AUAGUAGGUUUUGGGAGCUGGUUGACAAGGAAAGGAAUCUCCAUCUUGAAGGAAAGUGUGAAACAUGUGUGUACAAC
AUGAUGGGAAAAAGAGAGAAGAAGCUAGGGGAAUUCGGCAAGGCAAAAGGCAGCAGAGCCAUAUGGUACAUGUGGCU
UGGAGCACGCUUCUUAGAGUUUGAAGCCCUAGGAUUCUUAAAUGAAGAUCACUGGUUCUCCAGAGAGAACUCCCUGA
GUGGAGUGGAAGGAGAAGGGCUGCACAAGCUAGGUUACAUUCUAAGAGACGUGAGCAAGAAAGAGGGAGGAGCAAUG
UAUGCCGAUGACACCGCAGGAUGGGAUACAAAAAUCACACUAGAAGACCUAAAAAAUGAAGAGAUGGUAACAAACCA
CAUGGAAGGAGAACACAAGAAACUAGCCGAGGCCAUUUUCAAACUAACGUACCAAAACAAGGUGGUGCGUGUGCAAA
GACCAACACCAAGAGGCACAGUAAUGGACAUCAUAUCGAGAAGAGACCAAAGAGGUAGUGGACAAGUUGGCACCUAU
GGACUCAAUACUUUCACCAAUAUGGAAGCCCAACUAAUCAGACAGAUGGAGGGAGAAGGAGUCUUUAAAAGCAUUCA
GCACCUAACAAUCACAGAAGAAAUCGCUGUGCAAAACUGGUUAGCAAGAGUGGGGCGCGAAAGGUUAUCAAGAAUGG
CCAUCAGUGGAGAUGAUUGUGUUGUGAAACCUUUAGAUGACAGGUUCGCAAGCGCUUUAACAGCUCUAAAUGACAUG
GGAAAGAUUAGGAAAGACAUACAACAAUGGGAACCUUCAAGAGGAUGGAAUGAUUGGACACAAGUGCCCUUCUGUUC

ACACCAUUUCCAUGAGUUAAUCAUGAAAGACGGUCGCGUACUCGUUGUUCCAUGUAGAAACCAAGAUGAACUGAUUG
GCAGAGCCCGAAUCUCCCAAGGAGCAGGGUGGUCUUUGCGGGAGACGGCCUGUUUGGGGAAGUCUUACGCCCAAAUG
UGGAGCUUGAUGUACUUCCACAGACGCGACCUCAGGCUGGCGGCAAAUGCUAUUUGCUCGGCAGUACCAUCACAUUG
GGUUCCAACAAGUCGAACAACCUGGUCCAUACAUGCUAAACAUGAAUGGAUGACAACGGAAGACAUGCUGACAGUCU
GGAACAGGGUGUGGAUUCAAGAAAACCCAUGGAUGGAAGACAAAACUCCAGUGGAAACAUGGGAGGAAAUCCCAUAC
UUGGGGAAAAGAGAAGACCAAUGGUGCGGCUCAUUGAUUGGGUUAACAAGCAGGGCCACCUGGGCAAAGAACAUCCA
AGCAGCAAUAAAUCAAGUUAGAUCCCUUAUAGGCAAUGAAGAAUACACAGAUUACAUGCCAUCCAUGAAAAGAUUCA
GAAGAGAAGAGGAAGAAGCAGGAGUUCUGUGGUAGAAAGCAAAACUAACAUGAAACAAGGCUAGAAGUCAGGUCGGA
UUAAGCCAUAGUACGGAAAAAACUAUGCUACCUGUGAGCCCCGUCCAAGGACGUUAAAAGAAGUCAGGCCAUCAUAA
AUGCCAUAGCUUGAGUAAACUAUGCAGCCUGUAGCUCCACCUGAGAAGGUGUAAAAAAUCCGGGAGGCCACAAACCA
UGGAAGCUGUACGCAUGGCGUAGUGGACUAGCGGUUAGGGGAGACCCCUCCCUUACAAAUCGCAGCAACAAUGGGGG
CCCAAGGCGAGAUGAAGCUGUAGUCUCGCUGGAAGGACUAGAGGUUAGAGGAGACCCCCCCGAAACAAAAAACAGCA
UAUUGACGCUGGGAAAGACCAGAGAUCCUGCUGUCUCCUCAGCAUCAUUCCAGGCACAGAACGCCAGAAAAUGGAAU
GGUGCUGUUGAAUCAACAGGUUCU

>VDV2 prME核苷酸序列(SEQ ID NO: 25)

UUCCAUUUAACCACACGUAACGGAGAACCACACAUGAUCGUCAGCAGACAAGAGAAAGGGAAAAGUCUUCU
GUUUAAAACAGAGGUUGGCGUGAACAUGUGUACCCUCAUGGCCAUGGACCUUGGUGAAUUGUGUGAAGACACAAUC
ACGUACAAGUGUCCCCUUCUCAGGCAGAAUGAGCCAGAAGACAUAGACUGUUGGUGCAACUCUACGUCCACGUGGGU
AACUUAUGGGACGUGUACCACCAUGGGAGAACAUAGAAGAGAAAAAAGAUCAGUGGCACUCGUUCCACAUGUGCGAA
UGGGACUGGAGACACGAACUGAAACAUGGAUGUCAUCAGAAGGGGCCUGGAAACAUGUCCAGAGAAUUGAAACUUGG
AUCUUGAGACAUCCAGGCUUCACCAUGAUGGCAGCAAUCCUGGCAUACACCAUAGGAACGACACAUUUCCAAAGAGC
CCUGAUUUUCAUCUUACUGACAGCUGUCACUCCUUCAAUGACAAUGCGUUGCAUAGGAAUGUCAAAUAGAGACUUUG
UGGAAGGGGUUUCAGGAGGAAGCUGGGUUGACAUAGUCUUAGAACAUGGAAGCUGUGUGACGACGAUGGCAAAAAAC
AAACCAACAUUGGAUUUUGAACUGAUAAAAACAGAAGCCAAACAGCCUGCCACCCUAAGGAAGUACUGUAUAGAGGC
AAAGCUAACCAACACAACAACAGAAUCUCGCUGCCCAACACAAGGGGAACCCAGCCUAAAUGAAGAGCAGGACAAAA
GGUUCGUCUGCAAACACUCCAUGGUAGACAGAGGAUGGGGAAAUGGAUGUGGACUAUUUGGAAAGGGAGGCAUUGUG
ACCUGUGCUAUGUUCAGAUGCAAAAAGAACAUGGAAGGAAAAGUUGUGCAACCAGAAAACUUGGAAUACACCAUUGU
GAUAACACCUCACUCAGGGGAAGAGCAUGCAGUCGGAAAUGACACAGGAAAACAUGGCAAGGAAAUCAAAAUAACAC
CACAGAGUUCCAUCACAGAAGCAGAAUUGACAGGUUAUGGCACUGUCACAAUGGAGUGCUCUCCAAGAACGGGCCUC
GACUUCAAUGAGAUGGUGUUGCUGCAGAUGGAAAAUAAAGCUUGGCUGGUGCACAGGCAAUGGUUCCUAGACCUGCC
GUUACCAUGGUUGCCCGGAGCGGACACACAAGAGUCAAAUUGGAUACAGAAGGAGACAUUGGUCACUUUCAAAAAUC
CCCAUGCGAAGAAACAGGAUGUUGUUGUUUUAGGAUCCCAAGAAGGGGCCAUGCACACAGCACUUACAGGGGCCACA
GAAAUCCAAAUGUCAUCAGGAAACUUACUCUUCACAGGACAUCUCAAGUGCAGGCUGAGAAUGGACAAGCUACAGCU
CAAAGGAAUGUCAUACUCUAUGUGCACAGGAAAGUUUAAAGUUGUGAAGGAAAUAGCAGAAACACAACAUGGAACAA
UAGUUAUCAGAGUGCAAUAUGAAGGGGACGGCUCUCCAUGCAAGAUCCCUUUUGAGAUAAUGGAUUUGGAAAAAAGA
CAUGUCUUAGGUCGCCUGAUUACAGUCAACCCAAUUGUGACAGAAAAAGAUAGCCCAGUCAACAUAGAAGCAGAACC
UCCAUUUGGAGACAGCUACAUCAUCAUAGGAGUAGAGCCGGGACAACUGAAGCUCAACUGGUUUAAGAAAGGAAGUU
CUAUCGGCCAAAUGUUUGAGACAACAAUGAGGGGGGCGAAGAGAAUGGCCAUUUUAGGUGACACAGCCUGGGAUUUU
GGAUCCUUGGGAGGAGUGUUUACAUCUAUAGGAAAGGCUCUCCACCAAGUCUUUGGAGCAAUCUAUGGAGCUGCCUU

CAGUGGGGUUUCAUGGACUAUGAAAAUCCUCAUAGGAGUCAUUAUCACAUGGAUAGGAAUGAAUUCACGCAGCACCU
CACUGUCUGUGACACUAGUAUUGGUGGGAAUUGUGACACUGUAUUUGGGAGUCAUGGUGCAGGCC

>VDV2 E蛋白序列 (SEQ ID NO: 26)

MRCIGMSNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYCIEAKLTNTTTE
SRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFRCKKNMEGKVVQPENLEYTIVITPHSGE
EHAVGNDTGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGA
DTQESNWIQKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYSM
CTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTEKDSPVNIEAEPPFGDSYI
IIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTM
KILIGVIITWIGMNSRSTSLSVTLVLVGIVTLYLGVMVQA

>VDV2 M蛋白序列 (SEQ ID NO: 27)

SVALVPHVRMGLETRTETWMSSEGAWKHVQRIETWILRHPGFTMMAAILAYTIGTTHFQRALIFILLTAVTPS
MT

研究设计

在开放的随机对照 IIa 期试验中,在墨西哥城的两个中心招募 150 名年龄为 18-45 岁的健康成人,墨西哥城是登革热无病区。主要的排除标准为 :妊娠或哺乳、人免疫缺陷病毒、乙型或丙型肝炎血清阳性、免疫缺陷或可能干扰结果的任何其它慢性病、之前在高登革热地方流行性的地区居住或旅行 >2 周、黄病毒感染史或针对黄病毒病的既往接种。要求能够受孕的女性使用有效的避孕方法或在第 1 次注射前至少 4 周节欲直到最后一次注射后至少 4 周。

[0240] 参与者被随机分配到 2 组,在第 0 天和第 105 (±15 天) 天进行接种。各组接受下列制剂 :

第 1组:混合 CYD/VDV2 四价制剂,即包含 CYD-1、CYD-3、CYD4 和 VDV2 的制剂。

[0241] 第 2组:对照四价制剂 (CYD-TDV),即 CYD-1、CYD-2、CYD-3 和 CYD-4。

[0242] 制剂含有 10^5 $CCID_{50}$的 CYD 病毒的各个血清型,并将含有 10^4 $CCID_{50}$的 VDV-2 病毒的制剂给予第 1 组。

[0243] 病毒血症

为了评价疫苗的安全性,在每次注射后 7、14 和 21 天收集的血清中评价 CYD-1-4 或 VDV-2 的存在情况。通过 Global Clinical Immunology 实验室 (Sanofi Pasteur, Swiftwater, PA, USA) 进行分析。

[0244] 如之前 Poo 等, Pediatr Infect Dis J (2011) 30: e9 中的描述,分两步进行 CYD-1-4 病毒血症的分析。简单地说,第一,采用非血清型特异性的反转录酶 - 聚合酶链式反应 (RT-PCR) 以检测 4 种 CYD 病毒的任一种的存在。然后采用 4 个 CYD 血清型特异性定量 RT-PCR 对在该第一试验中呈阳性的样品进行分析。在非血清型特异性 RT-PCR 中,使用商用试剂盒,从血清中提取 RNA,并用来自黄热病核心基因序列的引物进行 RT-PCR。在血清型特异性 RT-PCR 中,再次使用商用试剂盒从血清中提取 RNA,针对各个血清型,用来自包膜非结构蛋白 1 连接基因序列的血清型特异性引物进行 RT-PCR。在第 1 组中针对血清型 2 进行登革热 RT-PCR,因为给予该组的四价混合制剂含有 VDV-2 病毒。

[0245] 免疫原性

通过对在每次注射后的 28 天以及在第 1 次注射后第 365 天收集的血清的 50% 蚀斑减少中和试验，测定 4 种登革热病毒血清型每一种的抗体水平。简单地说，将 2 倍系列稀释的热灭活的血清与恒定攻击剂量的各登革热血清型 DEN-1、-2、-3 或 -4 混合（表示为蚀斑形成单位 [PFU]/mL）。将混合物接种在汇合的 VERO 细胞单层的 24 孔板的各孔中。在接种几天后，通过蚀斑的形成表明登革热病毒感染。以血清的最高倒数稀释度 (1/dil) 计算中和抗体滴度，在之下观察到病毒蚀斑计数≥ 50% 减少 (PRNT50)。登革热 PRNT50 的定量下限为 10 ；滴度≥ 10 的样品被视为血清阳性。

[0246] 结果

在研究的第 0 天和第 105 天将制剂给予第 1 组和第 2 组的参与者。在第 1 次或第 2 次接种后就注射部位或全身反应原性而言，两组间没有显著差异。在每次注射后 7、14 和 21 天收集的血清中评价病毒血症（表 7）。在每次注射后的 28 天和在第 1 次注射后第 365 天，测量中和抗体滴度（表 8）。

[0247] 表 7. 在第 1次和第 2次注射后 7、4或 21天的疫苗病毒的病毒血症 (患有可检测的和可定量的病毒血症的 n (%))

	第 1 次注射		第 2 次注射	
	第 1 组 混合的 CYD/VDV	第 2 组 四价 CYD-TDV	第 1 组 混合的 CYD/VDV	第 2 组 四价 CYD-TDV
非血清型特异性				
N	29	31	28	29
可检测的病毒血症	27 (93%)	25 (81%)	1 (4%)	1 (3%)
可定量的病毒血症	1 (3%)	2 (6%)	0	0
DENV-1				
可检测的病毒血症	1 (3%)	4 (13%)	0	0
可定量的病毒血症	0	2 (7%)	0	0
DENV-2				
可检测的病毒血症	0	2 (6%)	0	0
可定量的病毒血症	0	0	0	0
DENV-3				
可检测的病毒血症	8 (28%)	7 (23%)	1 (4%)	0
可定量的病毒血症	0	0	0	0
DENV-4				
可检测的病毒血症	24 (83%)	21 (68%)	0	0
可定量的病毒血症	0	3 (1%)	0	0

在第 1 次注射之后，在两组参与者中以相似的比例观察到可检测的病毒血症，通过非血清型特异性 RT-PCR 试验测定（参见表 7）。在大部分的病例中，病毒血症低于定量下限。用血清型特异性测定法的分析表明，CYD-4 是最常检出的血清型，接着是 CYD-3。在第 1 组中在第 2 次注射混合 CYD/VDV 疫苗后或在第 2 组中注射 CYD-TDV 疫苗后，通过非血清型特异性测定法，只在每组一个参与者中检出病毒血症。

[0248] 因此，通过混合 CYD/VDV 和 CYD-TDV 诱导的病毒血症水平之间没有显著差异。

[0249] 表 8. 登革热抗体在第 1次和第 2次注射后 28天和在第 1次注射之后 365天的几

何平均滴度(95%置信区间)

	第1组 混合 CYD/VDV	第2组 CYD-TDV
第1次注射		
血清型1	15 (9; 28)	17 (10; 31)
血清型2	17 (8; 33)	32 (16; 65)
血清型3	64 (31; 133)	23 (13; 39)
血清型4	552 (299; 1019)	468 (226; 968)
第2次注射		
血清型1	54 (30; 96)	28 (15; 50)
血清型2	152 (79; 293)	43 (23; 79)
血清型3	127 (71; 229)	46 (29; 73)
血清型4	246 (159; 382)	173 (97; 307)
第1剂后365天		
血清型1	14 (9; 22)	18 (10; 30)
血清型2	55 (32; 94)	16 (9; 29)
血清型3	36 (20; 64)	11 (7; 16)
血清型4	103 (69; 155)	72 (44; 117)

从表8中可见,与CYD-TDV疫苗(第2组)相比,混合CYD/VDV疫苗(第1组)的第2次注射诱导针对登革热病毒的血清型2的更高的GMT。在第1剂后365天,在混合CYD/VDV组中还观察到对血清型2的反应改进。

[0250] 此外,当与接受CYD-TDV疫苗的组(第2组)相比时,混合CYD/VDV疫苗(第1组)的第2次注射导致针对登革热病毒的所有血清型的中和抗体反应改进。重要的是,与在第1次注射后第365天的CYD-TDV组相比,混合CYD/VDV制剂组显示针对登革热病毒更持久的中和抗体反应。

[0251] 因此实施例总体上表明,混合的CYD-1、3、4/VDV2疫苗制剂诱导针对登革热病毒血清型的免疫应答比CYD-TDV疫苗更强并更持久,同时显示类似的安全特征,如通过病毒血症水平所测定的。

[0252] 序列表

SEQ ID NO.	序列
1	prM+E CYD23传播性毒株核苷酸序列
2	prM+E CYD23传播性毒株蛋白质序列
3	prM+E共有血清型2蛋白质序列
4	prM+E LAV2核苷酸序列
5	prM+E BID/V585核苷酸序列
6	prM+E PR/DB023核苷酸序列
7	prM+E MD1280核苷酸序列
8	prM+E LAV2蛋白质序列
9	prM+E BID/V585蛋白质序列
10	prM+E PR/DB023蛋白质序列
11	prM+E MD1280蛋白质序列
12	E共有血清型2蛋白质序列
13	E LAV2蛋白质序列
14	E BID/V585蛋白质序列
15	E PR/DB023蛋白质序列

16	E MD1280 蛋白质序列
17	M 共有血清型 2 蛋白质序列
18	E CYD23 传播性毒株蛋白质序列
19	M LAV2 蛋白质序列
20	M BID/V585 蛋白质序列
21	M PR/DB023 蛋白质序列
22	M MD1280 蛋白质序列
23	M CYD23 传播性毒株蛋白质序列
24	VDV2 的完整核苷酸序列 (RNA 等同物)
25	prM+E VDV2 核苷酸序列 (RNA 等同物)
26	E VDV2 蛋白质序列
27	M VDV2 蛋白质序列

在所列出的核苷酸序列中，当核苷酸序列为 DNA 时，核苷酸 T 可被核苷酸 U 置换以得到 DNA 序列的 RNA 等同物。同样，当核苷酸序列为 RNA 时，核苷酸 U 可被核苷酸 T 置换以得到等同的 DNA 序列。上列 DNA 序列构成所述登革热病毒的 cDNA 序列，因此等同的 RNA 序列构成这些登革热病毒的正链 RNA。

[0001]

序列表

<110> Sanofi Pasteur

<120> 用于防止登革热病毒感染的疫苗组合物

<130> P39479WO

<150> EP12305911.5
<151> 2012-07-25

<150> EP12305908.1
<151> 2012-07-24

<160> 27

<170> PatentIn version 3.5

<210> 1
<211> 1983
<212> DNA
<213> 人共序列

<220>
<223> 临床试验传播毒株

<400> 1
ttccatctaa ccacacgcaa cggagaacca cacatgatcg tcggtataca ggagaaaggg 60

aaaagtcttc tgttcaaaac agaggatggt gtgaacatgt gcaccctcat ggctatggac 120

cttggtgaat tgtgtgaaga cacaatcacg tacaagtgtc ctcttctcag gcagaatgag 180

ccagaagaca tagactgttg gtgcaactcc acgtccacgt gggtaaccta tgggacctgt 240

accactacgg gagaacatag gagagaaaaa agatcagtgg cactcgttcc acatgtggga 300

atgggactgg agacgcgaac cgaaacatgg atgtcatcag aaggggcttg gaaacatgcc 360

cagagaattg aaacttggat cctgagacat ccaggcttca ccataatggc agcaatcctg 420

gcatacacca taggaacgac acatttccag agagtcctga ttttcatcct actgacagct 480

gtcgctcctt caatgacaat gcgttgcata ggaatatcaa atagagactt tgtagaaggg 540

[0002]

gtttcaggag gaagttgggt tgacatagtc ttagaacatg gaagctgtgt gacgacgatg 600

gcaaaaaaca aaccaacatt ggatttcgaa ctgataaaaa cggaagccaa acagcctgcc 660

accctaagga agtactgcat agaagcaaaa ctaaccaaca caacaacaga atcccgttgc 720

ccaacacaag gggaacccag cctaaaagaa gagcaggaca agaggttcgt ctgcaaacac 780

tccatggtag acagaggatg gggaaatgga tgtggattat ttggaaaggg aggcattgtg 840

acctgtgcta tgttcacatg caaaaagaac atggaaggga aaatcgtgca accagaaaac 900

ttggaataca ccattgtggt aacacctcac tcaggggaag agcatgcggt cggaaatgac 960

acaggaaaac acggcaagga aatcaaagta acaccacaga gttccatcac agaagcagaa 1020

ctgacaggtt atggcaccgt cacgatggag tgctccccga gaacaggcct cgacttcaat 1080

gagatggtgt tgctgcagat ggaaaataaa gcttggctgg tgcataggca atggtttcta 1140

gacctgccat taccatggct gcccggagcg gataaacaag aatcaaattg gatacagaaa 1200

gaaacattgg tcactttcaa aaatccccat gcgaagaaac aggatgttgt tgttttagga 1260

tcccaagaag gggccatgca tacagcactc acaggagcca cagaaatcca aatgtcgtca 1320

ggaaacttgc tcttcactgg acatctcaag tgcaggctga gaatggacaa gctacagctt 1380

aaaggaatgt catactctat gtgcacagga aagtttaaag ttgtgaagga aatagcagaa 1440

acacaacatg gaacgatagt tatcagagtg caatatgaag gggacggctc tccatgtaaa 1500

attccttttg agataatgga tttggaaaaa agatatgtct taggccgcct gatcacagtc 1560

aacccaattg taacagaaaa agacagccca gtcaacatag aagcagaacc tccattcgga 1620

gacagttaca tcatcatagg agtagagccg ggacaactga agctcaactg gttcaagaaa 1680

ggaagttcta tcggccaaat gtttgagaca acgatgagag gggcgaagag aatggccatt 1740

ttgggtgaca cagcctggga cttcggatcc ctgggaggag tgtttacatc tataggaaaa 1800

gctctccacc aagtctttgg agcgatctat ggggctgcct tcagtggggt ttcatggacc 1860

[0003]

atgaaaatcc tcataggagt cattatcaca tggataggaa tgaactcacg cagcacctca 1920

ctgtctgtgt cactggtact ggtgggaatt gtgacactgt atttaggagt catggtgcag 1980

gcc 1983

<210> 2
<211> 661
<212> PRT
<213> 人共序列

<220>
<223> 临床试验传播毒株

<400> 2

Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val Gly Ile
1 5 10 15

Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Asp Gly Val Asn
20 25 30

Met Cys Thr Leu Met Ala Met Asp Leu Gly Glu Leu Cys Glu Asp Thr
35 40 45

Ile Thr Tyr Lys Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu Asp Ile
50 55 60

Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly Thr Cys
65 70 75 80

Thr Thr Thr Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala Leu Val
85 90 95

Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp Met Ser
100 105 110

[0004]

Ser Glu Gly Ala Trp Lys His Ala Gln Arg Ile Glu Thr Trp Ile Leu
115 120 125

Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile Leu Ala Tyr Thr Ile
130 135 140

Gly Thr Thr His Phe Gln Arg Val Leu Ile Phe Ile Leu Leu Thr Ala
145 150 155 160

Val Ala Pro Ser Met Thr Met Arg Cys Ile Gly Ile Ser Asn Arg Asp
165 170 175

Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val Leu Glu
180 185 190

His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr Leu Asp
195 200 205

Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys
210 215 220

Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys
225 230 235 240

Pro Thr Gln Gly Glu Pro Ser Leu Lys Glu Glu Gln Asp Lys Arg Phe
245 250 255

Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly Cys Gly
260 265 270

Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Thr Cys Lys
275 280 285

[0005]

Lys Asn Met Glu Gly Lys Ile Val Gln Pro Glu Asn Leu Glu Tyr Thr
290 295 300

Ile Val Val Thr Pro His Ser Gly Glu Glu His Ala Val Gly Asn Asp
305 310 315 320

Thr Gly Lys His Gly Lys Glu Ile Lys Val Thr Pro Gln Ser Ser Ile
325 330 335

Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu Cys Ser
340 345 350

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln Met Glu
355 360 365

Asn Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu Pro Leu
370 375 380

Pro Trp Leu Pro Gly Ala Asp Lys Gln Glu Ser Asn Trp Ile Gln Lys
385 390 395 400

Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln Asp Val
405 410 415

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
420 425 430

Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr Gly His
435 440 445

Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly Met Ser
450 455 460

[0006]

Tyr Ser Met Cys Thr Gly Lys Phe Lys Val Val Lys Glu Ile Ala Glu
465 470 475 480

Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly Asp Gly
485 490 495

Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys Arg Tyr
500 505 510

Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu Lys Asp
515 520 525

Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile
530 535 540

Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys
545 550 555 560

Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly Ala Lys
565 570 575

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly
580 585 590

Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe Gly Ala
595 600 605

Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys Ile Leu
610 615 620

Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser Thr Ser
625 630 635 640

[0007]

Leu Ser Val Ser Leu Val Leu Val Gly Ile Val Thr Leu Tyr Leu Gly
645 650 655

Val Met Val Gln Ala
660

<210> 3
<211> 661
<212> PRT
<213> 人共序列

<220>
<223> prM+E 通用血清型 2

<220>
<221> 变体
<222> (295)..(295)
<223> Xaa 可以是 Val 或 Ile

<220>
<221> 变体
<222> (474)..(474)
<223> Xaa 可以是 Val 或 Ile

<400> 3

Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val Gly Arg
1 5 10 15

Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Asp Gly Val Asn
20 25 30

Met Cys Thr Leu Met Ala Ile Asp Leu Gly Glu Leu Cys Glu Asp Thr
35 40 45

Ile Thr Tyr Lys Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu Asp Ile

[0008]

50 55 60

Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly Thr Cys
65 70 75 80

Thr Thr Thr Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala Leu Val
85 90 95

Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp Met Ser
100 105 110

Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp Ile Leu
115 120 125

Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile Leu Ala Tyr Thr Ile
130 135 140

Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu Thr Ala
145 150 155 160

Val Ala Pro Ser Met Thr Met Arg Cys Ile Gly Ile Ser Asn Arg Asp
165 170 175

Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val Leu Glu
180 185 190

His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr Leu Asp
195 200 205

Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys
210 215 220

Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys

[0009]

225 230 235 240

Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe
245 250 255

Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly Cys Gly
260 265 270

Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Thr Cys Lys
275 280 285

Lys Asn Met Glu Gly Lys Xaa Val Gln Pro Glu Asn Leu Glu Tyr Thr
290 295 300

Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly Asn Asp
305 310 315 320

Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile
325 330 335

Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu Cys Ser
340 345 350

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln Met Glu
355 360 365

Asp Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu Pro Leu
370 375 380

Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys
385 390 395 400

Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln Asp Val

[0010]

405 410 415

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
420 425 430

Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr Gly His
435 440 445

Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly Met Ser
450 455 460

Tyr Ser Met Cys Thr Gly Lys Phe Lys Xaa Val Lys Glu Ile Ala Glu
465 470 475 480

Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly Asp Gly
485 490 495

Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys Arg His
500 505 510

Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu Lys Asp
515 520 525

Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile
530 535 540

Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys
545 550 555 560

Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly Ala Lys
565 570 575

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly

[0011]

580 585 590

Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe Gly Ala
595 600 605

Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys Ile Leu
610 615 620

Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser Thr Ser
625 630 635 640

Leu Ser Val Ser Leu Val Leu Val Gly Val Val Thr Leu Tyr Leu Gly
645 650 655

Val Met Val Gln Ala
660

<210> 4
<211> 1983
<212> DNA
<213> 人共序列

<220>
<223> prM+E LAV2

<400> 4
ttccatttaa ccacacgtaa cggagaacca cacatgatcg tcagcagaca agagaaaggg 60

aaaagtcttc tgtttaaaac agaggttggc gtgaacatgt gtaccctcat ggccatggac 120

cttggtgaat tgtgtgaaga cacaatcacg tacaagtgtc cccttctcag gcagaatgag 180

ccagaagaca tagactgttg gtgcaactct acgtccacgt gggtaactta tgggacgtgt 240

accaccatgg gagaacatag aagagaaaaa agatcagtgg cactcgttcc acatgtggga 300

atgggactgg agacacgaac tgaaacatgg atgtcatcag aaggggcctg gaaacatgtc 360

[0012]

cagagaattg aaacttggat cttgagacat ccaggcttca ccatgatggc agcaatcctg 420

gcatacacca taggaacgac acatttccaa agagccctga ttttcatctt actgacagct 480

gtcactcctt caatgacaat gcgttgcata ggaatgtcaa atagagactt tgtggaaggg 540

gtttcaggag gaagctgggt tgacatagtc ttagaacatg gaagctgtgt gacgacgatg 600

gcaaaaaaca aaccaacatt ggattttgaa ctgataaaaa cagaagccaa acagcctgcc 660

accctaagga agtactgtat agaggcaaag ctaaccaaca caacaacaga atctcgctgc 720

ccaacacaag gggaacccag cctaaatgaa gagcaggaca aaaggttcgt ctgcaaacac 780

tccatggtag acagaggatg gggaaatgga tgtggactat ttggaaaggg aggcattgtg 840

acctgtgcta tgttcagatg caaaaagaac atggaaggaa aagttgtgca accagaaaac 900

ttggaataca ccattgtgat aacacctcac tcaggggaag agcatgcagt cggaaatgac 960

acaggaaaac atggcaagga aatcaaaata acaccacaga gttccatcac agaagcagaa 1020

ttgacaggtt atggcactgt cacaatggag tgctctccaa gaacgggcct cgacttcaat 1080

gagatggtgt tgctgcagat ggaaaataaa gcttggctgg tgcacaggca atggttccta 1140

gacctgccgt taccatggtt gcccggagcg gacacacaag ggtcaaattg gatacagaaa 1200

gagacattgg tcactttcaa aaatccccat gcgaagaaac aggatgttgt tgttttagga 1260

tcccaagaag gggccatgca cacagcactt acaggggcca cagaaatcca aatgtcatca 1320

ggaaacttac tcttcacagg acatctcaag tgcaggctga gaatggacaa gctacagctc 1380

aaaggaatgt catactctat gtgcacagga aagtttaaag ttgtgaagga aatagcagaa 1440

acacaacatg gaacaatagt tatcagagtg caatatgaag gggacggctc tccatgcaag 1500

atccctttg agataatgga tttggaaaaa agacatgtct taggtcgcct gattacagtc 1560

aacccaattg tgacagaaaa agatagccca gtcaacatag aagcagaacc tccatttgga 1620

gacagctaca tcatcatagg agtagagccg ggacaactga agctcaactg gtttaagaaa 1680

[0013]

ggaagttcta tcggccaaat gtttgagaca acaatgaggg gggcgaagag aatggccatt 1740

ttaggtgaca cagcctggga ttttggatcc ttgggaggag tgtttacatc tataggaaag 1800

gctctccacc aagtctttgg agcaatctat ggagctgcct tcagtggggt ttcatggact 1860

atgaaaatcc tcataggagt cattatcaca tggataggaa tgaattcacg cagcacctca 1920

ctgtctgtga cactagtatt ggtgggaatt gtgacactgt atttgggagt catggtgcag 1980

gcc 1983

<210> 5
<211> 1983
<212> DNA
<213> 人共序列

<220>
<223> prM+E BID/V585

<400> 5
ttccatttaa ccacacgtaa tggagaacca cacatgatcg ttggtaggca agagaaaggg 60

aaaagtcttc tgtttaaaac agaggatggt gttaacatgt gcaccctcat ggccatagac 120

cttggtgaat tgtgtgaaga tacaatcacg tacaagtgcc ccctcctcag gcaaaatgaa 180

ccagaagaca tagattgttg gtgcaactct acgtccacat gggtaactta tgggacatgt 240

accaccacag gagaacacag aagagaaaaa agatcagtgg cactcgttcc acatgtgggc 300

atgggactgg agacacgaac tgaaacatgg atgtcatcag aaggggcctg gaaacatgtt 360

cagagaattg aaacctggat cttgagacat ccaggcttta ccataatggc agcaatcctg 420

gcatatacca taggaacgac acatttccaa agggctctga tcttcatttt actgacagcc 480

gttgctcctt caatgacaat gcgttgcata ggaatatcaa atagagactt cgtagaaggg 540

gtttcaggag gaagttgggt tgacatagtc ttagaacatg gaagttgtgt gacgacgatg 600

gcaaaaaata aaccaacatt ggattttgaa ctgataaaaa cagaagccaa acaacctgcc 660

[0014]

actctaagga agtactgtat agaagcaaag ctgaccaata caacaacaga atctcgttgc 720

ccaacacaag gggaacccag tctaaatgaa gagcaggaca aaaggttcat ctgcaaacac 780

tccatggtag acagaggatg gggaaatgga tgtggattat ttggaaaggg aggcattgtg 840

acctgtgcta tgttcacatg caaaaagaac atggaaggaa aagtcgtgca gccagaaaat 900

ctggaataca ccatcgtgat aacacctcac tcaggagaag agcacgctgt aggtaatgac 960

acaggaaagc atggcaagga aatcaaaata acaccacaga gctccatcac agaagcagaa 1020

ctgacaggct atggcactgt cacgatggag tgctctccga gaacgggcct cgacttcaat 1080

gagatggtac tgctgcagat ggaagacaaa gcttggctgg tgcacaggca atggttccta 1140

gacctgccgt taccatggct acccggagcg gacacacaag gatcaaattg gatacagaaa 1200

gagacgttgg tcactttcaa aaatccccac gcgaagaaac aggacgtcgt tgttttagga 1260

tctcaagaag gggccatgca cacggcactt acaggggcca cagaaatcca gatgtcatca 1320

ggaaacttac tgttcacagg acatctcaag tgtaggctga gaatggacaa attacagctt 1380

aaaggaatgt catactctat gtgtacagga aagtttaaaa ttgtgaagga aatagcagaa 1440

acacaacatg gaacaatagt tatcagagta caatatgaag gggacggctc tccatgtaag 1500

attccttttg agataatgga tttggaaaaa agacacgtcc taggtcgcct gattacagtg 1560

aacccaatcg taacagaaaa agatagccca gtcaacatag aagcagaacc tccattcgga 1620

gacagctaca tcatcatagg agtagagccg ggacaattga aactcaattg gttcaagaag 1680

ggaagttcca ttggccaaat gtttgagaca acaatgagag gagcgaagag aatggccatt 1740

ttaggtgaca cagcctggga ttttggatcc ctgggaggag tgtttacatc tataggaaag 1800

gctctccacc aagttttcgg agcaatctat ggggctgctt ttagtggggt ctcatggact 1860

atgaaaatcc tcataggagt tattatcaca tggataggaa tgaattcacg tagcacctca 1920

ctgtctgtgt cactagtatt ggtgggagtc gtgacactgt acttgggggt tatggtgcag 1980

[0015]

gct 1983

<210> 6
<211> 1983
<212> DNA
<213> 人共序列

<220>
<223> prM+E PR/DB023

<400> 6
ttccatttaa ccacacgtaa tggagaacca cacatgatcg ttggtaggca agagaaaggg 60

aaaagtcttc tgttcaaaac agaggatggt gttaacatgt gtaccctcat ggccatagac 120

cttggtgaat tgtgtgaaga tacaatcacg tacaagtgcc ccctcctcag gcaaaatgaa 180

ccagaagaca tagattgttg gtgcaactct acgtccacat gggtaactta tgggacatgt 240

accaccacag gagaacacag aagagaaaaa agatcagtgg cactcgttcc acatgtgggc 300

atgggactgg agacacgaac tgaaacatgg atgtcatcag aaggggcctg gaaacatgtt 360

cagagaattg aaacctggat attgagacat ccaggcttta ccataatggc agcaatcctg 420

gcatatacca taggaacgac acatttccaa agggctctga tcttcatttt actgacagcc 480

gtcgctcctt caatgacaat gcgttgcata ggaatatcaa atagagactt cgtagaaggg 540

gtttcaggag gaagttgggt tgacatagtc ttagaacatg gaagttgtgt gacgacgatg 600

gcaaaaaata aaccaacatt ggattttgaa ctgataaaaa cagaagccaa acaacctgcc 660

actctaagga agtactgtat agaagcaaag ctgaccaata caacaacaga atctcgttgc 720

ccaacacaag gggaacccag tctaaatgaa gagcaggaca aaaggttcat ctgcaaacac 780

tccatggtag acagaggatg gggaaatgga tgtggattat ttggaaaagg aggcattgta 840

acctgtgcta tgttcacatg caaaaagaac atggaaggaa aagttgtgct gccagaaaat 900

ctggaataca ccatcgtgat aacacctcac tcaggagaag agcacgctgt aggtaatgac 960

[0016]

acaggaaaac atggcaagga aattaaaata acaccacaga gttccatcac agaagcagaa 1020

ctgacaggct atggcactgt cacgatggag tgctctccga gaacgggcct cgacttcaat 1080

gagatggtgc tgctgcagat ggaagacaaa gcctggctgg tgcacaggca atggttccta 1140

gatctgccgt taccatggct acccggagcg gacacacaag gatcaaattg gatacagaaa 1200

gagacgttgg tcactttcaa aaatccccac gcgaagaaac aggacgtcgt tgttttagga 1260

tctcaagaag gggccatgca cacggcactt acaggggcca cagaaatcca gatgtcatca 1320

ggaaacttac tgttcacagg acatctcaag tgtaggctga gaatggacaa attacagctt 1380

aaaggaatgt catactctat gtgtacagga aagtttaaaa ttgtgaagga aatagcagaa 1440

acacaacatg gaacaatagt tatcagagta caatatgaag gggacggctc tccatgtaag 1500

attccttttg agataatgga tttagaaaaa agacacgtcc taggtcgcct gattacagtg 1560

aacccaatcg taacagaaaa agatagccca gtcaacatag aagcagaacc tccattcgga 1620

gacagctaca tcatcatagg agtagagccg ggacaattga aactcaattg gttcaagaag 1680

ggaagttcca ttggccaaat gtttgagaca acaatgagag gagcgaagag aatggccatt 1740

ttaggtgaca cagcctggga ttttggatcc ctgggaggag tgtttacatc tataggaaag 1800

gctctccacc aagttttcgg agcaatctat ggggctgctt ttagtggggt ctcatggact 1860

atgaaaatcc tcataggagt tatcatcaca tggataggaa tgaattcacg tagcacctca 1920

ctgtctgtgt cactagtatt ggtgggagtc gtgacactgt acttgggggt tatggtgcag 1980

gct 1983

<210> 7
<211> 1983
<212> DNA
<213> 人共序列

<220>
<223> prM+E MD1280

[0017]

<400> 7

ttccatttaa ccacacgaaa tggagaacca cacatgatcg ttggcagaca agagaaaggg 60

aaaagccttc tgtttaaaac agaggatggt gtgaacatgt gtaccctcat ggccattgat 120

cttggtgaat tgtgtgaaga tacaatcacg tacaagtgcc ccctcctcag gcagaatgaa 180

ccagaagata tagattgttg gtgcaactcc acgtccacat gggtaactta tgggacgtgt 240

accaccacag gagaacacag aagagaaaaa agatcagtgg cactcgttcc acatgtgggt 300

atgggactgg agacacgaac tgaaacatgg atgtcgtcag aaggggcctg gaaacacgct 360

cagagaattg aaacttggat cttgagacat ccaggcttta ccataatggc agcaatcctg 420

gcatataccg taggaacgac acatttccaa agggccctga ttttcatctt actggcagct 480

gtcgctcctt caatgacaat gcgttgcata ggaatatcaa atagagactt tgtagaaggg 540

gtttcaggag gaagctgggt tgacatagtc ttagaacatg gaagttgtgt gacgacaatg 600

gcaaaaaata aaccaacact ggattttgaa ctgataaaaa cagaagccaa acaacctgcc 660

actctaagga agtactgtat agaggcaaag ctgaccaata caacaacaga atctcgttgc 720

ccaacacaag gggaacccag tctaaatgaa gagcaggaca aaaggttcgt ctgcaaacac 780

tccatggtag acagaggatg gggaaatgga tgtggattat ttggaaaggg aggcattgtg 840

acctgtgcta tgttcacatg caaaaagaac atggaaggaa aaatcgtgca accagaaaat 900

ttggaataca ccatcgtgat aacacctcac tcaggagaag agcacgctgt aggtaatgac 960

acaggaaaac atggtaagga aattaaaata acaccacaga gttccatcac agaagcagaa 1020

ctgacaggct atggcacagt cacgatggag tgctctccga gaacgggcct tgacttcaat 1080

gagatggtgc tgctgcagat ggaagataaa gcttggctgg tgcacaggca atggttccta 1140

gacctgccgt taccatggct acccggagcg gacacacaag gatcaaattg gatacagaaa 1200

gagacattgg tcactttcaa aaatccccac gcgaagaagc aggatgtcgt tgttttagga 1260

[0018]

tctcaagaag gagccatgca cacggcactc acaggggcca cagaaatcca gatgtcatca 1320

ggaaacttac tattcacagg acatctcaaa tgcaggctga gaatggacaa actacagctc 1380

aaaggaatgt catactctat gtgtacagga aagtttaaaa ttgtgaagga aatagcagaa 1440

acacaacatg gaacaatagt tatcagagta caatatgaag gagacggctc tccatgtaag 1500

atccctttg aaataatgga tttggaaaaa agacatgtct taggtcgcct gattacagtt 1560

aatccgatcg taacagaaaa agatagccca gtcaacatag aagcagaacc tccattcgga 1620

gacagctaca tcattatagg agtagagccg ggacaattga aactcaactg gttcaagaaa 1680

ggaagttcca tcggccaaat gtttgagacg acaatgagag gagcaaagag aatggccatt 1740

ttaggtgaca cagcctggga ttttggatct ctgggaggag tgtttacatc tataggaaag 1800

gctctccacc aagttttcgg agcaatctat ggggctgcct ttagtggggt ttcatggact 1860

atgaaaatcc tcataggagt catcatcaca tggataggaa tgaattcacg tagcacctca 1920

ctgtctgtgt cactagtatt ggtgggaatc ataacactgt acttgggagc tatggtgcag 1980

gct 1983

<210> 8
<211> 661
<212> PRT
<213> 人共序列

<220>
<223> prM+E LAV2

<400> 8

Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val Ser Arg
1 5 10 15

Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Val Gly Val Asn
20 25 30

[0019]

Met Cys Thr Leu Met Ala Met Asp Leu Gly Glu Leu Cys Glu Asp Thr
35 40 45

Ile Thr Tyr Lys Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu Asp Ile
50 55 60

Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly Thr Cys
65 70 75 80

Thr Thr Met Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala Leu Val
85 90 95

Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp Met Ser
100 105 110

Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp Ile Leu
115 120 125

Arg His Pro Gly Phe Thr Met Met Ala Ala Ile Leu Ala Tyr Thr Ile
130 135 140

Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu Thr Ala
145 150 155 160

Val Thr Pro Ser Met Thr Met Arg Cys Ile Gly Met Ser Asn Arg Asp
165 170 175

Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val Leu Glu
180 185 190

His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr Leu Asp
195 200 205

[0020]

Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys
210 215 220

Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys
225 230 235 240

Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe
245 250 255

Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly Cys Gly
260 265 270

Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Arg Cys Lys
275 280 285

Lys Asn Met Glu Gly Lys Val Val Gln Pro Glu Asn Leu Glu Tyr Thr
290 295 300

Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly Asn Asp
305 310 315 320

Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile
325 330 335

Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu Cys Ser
340 345 350

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln Met Glu
355 360 365

Asn Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu Pro Leu
370 375 380

[0021]

Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys
385 390 395 400

Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln Asp Val
405 410 415

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
420 425 430

Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr Gly His
435 440 445

Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly Met Ser
450 455 460

Tyr Ser Met Cys Thr Gly Lys Phe Lys Val Val Lys Glu Ile Ala Glu
465 470 475 480

Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly Asp Gly
485 490 495

Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys Arg His
500 505 510

Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu Lys Asp
515 520 525

Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile
530 535 540

Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys
545 550 555 560

[0022]

Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly Ala Lys
565 570 575

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly
580 585 590

Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe Gly Ala
595 600 605

Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys Ile Leu
610 615 620

Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser Thr Ser
625 630 635 640

Leu Ser Val Thr Leu Val Leu Val Gly Ile Val Thr Leu Tyr Leu Gly
645 650 655

Val Met Val Gln Ala
660

<210> 9
<211> 661
<212> PRT
<213> 人共序列

<220>
<223> prM+E BID/V585

<400> 9

Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val Gly Arg
1 5 10 15

Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Asp Gly Val Asn

[0023]

20 25 30

Met Cys Thr Leu Met Ala Ile Asp Leu Gly Glu Leu Cys Glu Asp Thr
35 40 45

Ile Thr Tyr Lys Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu Asp Ile
50 55 60

Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly Thr Cys
65 70 75 80

Thr Thr Thr Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala Leu Val
85 90 95

Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp Met Ser
100 105 110

Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp Ile Leu
115 120 125

Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile Leu Ala Tyr Thr Ile
130 135 140

Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu Thr Ala
145 150 155 160

Val Ala Pro Ser Met Thr Met Arg Cys Ile Gly Ile Ser Asn Arg Asp
165 170 175

Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val Leu Glu
180 185 190

His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr Leu Asp

[0024]

195 200 205

Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys
210 215 220

Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys
225 230 235 240

Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe
245 250 255

Ile Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly Cys Gly
260 265 270

Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Thr Cys Lys
275 280 285

Lys Asn Met Glu Gly Lys Val Val Gln Pro Glu Asn Leu Glu Tyr Thr
290 295 300

Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly Asn Asp
305 310 315 320

Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile
325 330 335

Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu Cys Ser
340 345 350

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln Met Glu
355 360 365

Asp Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu Pro Leu

[0025]

370 375 380

Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys
385 390 395 400

Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln Asp Val
405 410 415

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
420 425 430

Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr Gly His
435 440 445

Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly Met Ser
450 455 460

Tyr Ser Met Cys Thr Gly Lys Phe Lys Ile Val Lys Glu Ile Ala Glu
465 470 475 480

Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly Asp Gly
485 490 495

Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys Arg His
500 505 510

Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu Lys Asp
515 520 525

Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile
530 535 540

Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys

[0026]

545 550 555 560

Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly Ala Lys
565 570 575

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly
580 585 590

Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe Gly Ala
595 600 605

Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys Ile Leu
610 615 620

Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser Thr Ser
625 630 635 640

Leu Ser Val Ser Leu Val Leu Val Gly Val Val Thr Leu Tyr Leu Gly
645 650 655

Val Met Val Gln Ala
660

<210> 10
<211> 661
<212> PRT
<213> 人共序列

<220>
<223> prM+E PR/DB023

<400> 10

Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val Gly Arg
1 5 10 15

[0027]

Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Asp Gly Val Asn
20 25 30

Met Cys Thr Leu Met Ala Ile Asp Leu Gly Glu Leu Cys Glu Asp Thr
35 40 45

Ile Thr Tyr Lys Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu Asp Ile
50 55 60

Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly Thr Cys
65 70 75 80

Thr Thr Thr Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala Leu Val
85 90 95

Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp Met Ser
100 105 110

Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp Ile Leu
115 120 125

Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile Leu Ala Tyr Thr Ile
130 135 140

Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu Thr Ala
145 150 155 160

Val Ala Pro Ser Met Thr Met Arg Cys Ile Gly Ile Ser Asn Arg Asp
165 170 175

Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val Leu Glu
180 185 190

[0028]

```
His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr Leu Asp
        195                 200                 205

Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys
    210                 215                 220

Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys
225                 230                 235                 240

Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe
                245                 250                 255

Ile Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly Cys Gly
            260                 265                 270

Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Thr Cys Lys
        275                 280                 285

Lys Asn Met Glu Gly Lys Val Val Leu Pro Glu Asn Leu Glu Tyr Thr
    290                 295                 300

Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly Asn Asp
305                 310                 315                 320

Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile
                325                 330                 335

Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu Cys Ser
            340                 345                 350

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln Met Glu
        355                 360                 365
```

[0029]

Asp Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu Pro Leu
370 375 380

Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys
385 390 395 400

Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln Asp Val
405 410 415

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
420 425 430

Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr Gly His
435 440 445

Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly Met Ser
450 455 460

Tyr Ser Met Cys Thr Gly Lys Phe Lys Ile Val Lys Glu Ile Ala Glu
465 470 475 480

Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly Asp Gly
485 490 495

Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys Arg His
500 505 510

Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu Lys Asp
515 520 525

Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile
530 535 540

[0030]

Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys
545 550 555 560

Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly Ala Lys
565 570 575

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly
580 585 590

Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe Gly Ala
595 600 605

Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys Ile Leu
610 615 620

Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser Thr Ser
625 630 635 640

Leu Ser Val Ser Leu Val Leu Val Gly Val Val Thr Leu Tyr Leu Gly
645 650 655

Val Met Val Gln Ala
660

<210> 11
<211> 661
<212> PRT
<213> 人共序列

<220>
<223> prM+E MD1280

<400> 11

Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val Gly Arg

[0031]

1 5 10 15

Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Asp Gly Val Asn
20 25 30

Met Cys Thr Leu Met Ala Ile Asp Leu Gly Glu Leu Cys Glu Asp Thr
35 40 45

Ile Thr Tyr Lys Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu Asp Ile
50 55 60

Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly Thr Cys
65 70 75 80

Thr Thr Thr Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala Leu Val
85 90 95

Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp Met Ser
100 105 110

Ser Glu Gly Ala Trp Lys His Ala Gln Arg Ile Glu Thr Trp Ile Leu
115 120 125

Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile Leu Ala Tyr Thr Val
130 135 140

Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu Ala Ala
145 150 155 160

Val Ala Pro Ser Met Thr Met Arg Cys Ile Gly Ile Ser Asn Arg Asp
165 170 175

Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val Leu Glu

[0032]

180 185 190

His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr Leu Asp
195 200 205

Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys
210 215 220

Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys
225 230 235 240

Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe
245 250 255

Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly Cys Gly
260 265 270

Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Thr Cys Lys
275 280 285

Lys Asn Met Glu Gly Lys Ile Val Gln Pro Glu Asn Leu Glu Tyr Thr
290 295 300

Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly Asn Asp
305 310 315 320

Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile
325 330 335

Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu Cys Ser
340 345 350

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln Met Glu

[0033]

355 360 365

Asp Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu Pro Leu
370 375 380

Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys
385 390 395 400

Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln Asp Val
405 410 415

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
420 425 430

Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr Gly His
435 440 445

Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly Met Ser
450 455 460

Tyr Ser Met Cys Thr Gly Lys Phe Lys Ile Val Lys Glu Ile Ala Glu
465 470 475 480

Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly Asp Gly
485 490 495

Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys Arg His
500 505 510

Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu Lys Asp
515 520 525

Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile

[0034]

530 535 540

Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys
545 550 555 560

Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly Ala Lys
565 570 575

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly
580 585 590

Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe Gly Ala
595 600 605

Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys Ile Leu
610 615 620

Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser Thr Ser
625 630 635 640

Leu Ser Val Ser Leu Val Leu Val Gly Ile Ile Thr Leu Tyr Leu Gly
645 650 655

Ala Met Val Gln Ala
660

<210> 12
<211> 495
<212> PRT
<213> 人共序列

<220>
<223> E 通用

[0035]

<220>
<221> 变体
<222> (129)..(129)
<223> Xaa 可以是 Val 或 Ile

<220>
<221> 变体
<222> (308)..(308)
<223> Xaa 可以是 Val 或 Ile

<400> 12

Met Arg Cys Ile Gly Ile Ser Asn Arg Asp Phe Val Glu Gly Val Ser
1 5 10 15

Gly Gly Ser Trp Val Asp Ile Val Leu Glu His Gly Ser Cys Val Thr
20 25 30

Thr Met Ala Lys Asn Lys Pro Thr Leu Asp Phe Glu Leu Ile Lys Thr
35 40 45

Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys Tyr Cys Ile Glu Ala Lys
50 55 60

Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys Pro Thr Gln Gly Glu Pro
65 70 75 80

Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe Val Cys Lys His Ser Met
85 90 95

Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Gly
100 105 110

Ile Val Thr Cys Ala Met Phe Thr Cys Lys Lys Asn Met Glu Gly Lys
115 120 125

[0036]

Xaa Val Gln Pro Glu Asn Leu Glu Tyr Thr Ile Val Ile Thr Pro His
130 135 140

Ser Gly Glu Glu His Ala Val Gly Asn Asp Thr Gly Lys His Gly Lys
145 150 155 160

Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile Thr Glu Ala Glu Leu Thr
165 170 175

Gly Tyr Gly Thr Val Thr Met Glu Cys Ser Pro Arg Thr Gly Leu Asp
180 185 190

Phe Asn Glu Met Val Leu Leu Gln Met Glu Asp Lys Ala Trp Leu Val
195 200 205

His Arg Gln Trp Phe Leu Asp Leu Pro Leu Pro Trp Leu Pro Gly Ala
210 215 220

Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys Glu Thr Leu Val Thr Phe
225 230 235 240

Lys Asn Pro His Ala Lys Lys Gln Asp Val Val Val Leu Gly Ser Gln
245 250 255

Glu Gly Ala Met His Thr Ala Leu Thr Gly Ala Thr Glu Ile Gln Met
260 265 270

Ser Ser Gly Asn Leu Leu Phe Thr Gly His Leu Lys Cys Arg Leu Arg
275 280 285

Met Asp Lys Leu Gln Leu Lys Gly Met Ser Tyr Ser Met Cys Thr Gly
290 295 300

[0037]

Lys Phe Lys Xaa Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile
305 310 315 320

Val Ile Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro
325 330 335

Phe Glu Ile Met Asp Leu Glu Lys Arg His Val Leu Gly Arg Leu Ile
340 345 350

Thr Val Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu
355 360 365

Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro
370 375 380

Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln
385 390 395 400

Met Phe Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly
405 410 415

Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly Gly Val Phe Thr Ser Ile
420 425 430

Gly Lys Ala Leu His Gln Val Phe Gly Ala Ile Tyr Gly Ala Ala Phe
435 440 445

Ser Gly Val Ser Trp Thr Met Lys Ile Leu Ile Gly Val Ile Ile Thr
450 455 460

Trp Ile Gly Met Asn Ser Arg Ser Thr Ser Leu Ser Val Ser Leu Val
465 470 475 480

[0038]

Leu Val Gly Val Val Thr Leu Tyr Leu Gly Val Met Val Gln Ala
485 490 495

<210> 13
<211> 495
<212> PRT
<213> 人共序列

<220>
<223> E LAV2

<400> 13

Met Arg Cys Ile Gly Met Ser Asn Arg Asp Phe Val Glu Gly Val Ser
1 5 10 15

Gly Gly Ser Trp Val Asp Ile Val Leu Glu His Gly Ser Cys Val Thr
20 25 30

Thr Met Ala Lys Asn Lys Pro Thr Leu Asp Phe Glu Leu Ile Lys Thr
35 40 45

Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys Tyr Cys Ile Glu Ala Lys
50 55 60

Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys Pro Thr Gln Gly Glu Pro
65 70 75 80

Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe Val Cys Lys His Ser Met
85 90 95

Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Gly
100 105 110

Ile Val Thr Cys Ala Met Phe Arg Cys Lys Lys Asn Met Glu Gly Lys
115 120 125

[0039]

Val Val Gln Pro Glu Asn Leu Glu Tyr Thr Ile Val Ile Thr Pro His
130 135 140

Ser Gly Glu Glu His Ala Val Gly Asn Asp Thr Gly Lys His Gly Lys
145 150 155 160

Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile Thr Glu Ala Glu Leu Thr
165 170 175

Gly Tyr Gly Thr Val Thr Met Glu Cys Ser Pro Arg Thr Gly Leu Asp
180 185 190

Phe Asn Glu Met Val Leu Leu Gln Met Glu Asn Lys Ala Trp Leu Val
195 200 205

His Arg Gln Trp Phe Leu Asp Leu Pro Leu Pro Trp Leu Pro Gly Ala
210 215 220

Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys Glu Thr Leu Val Thr Phe
225 230 235 240

Lys Asn Pro His Ala Lys Lys Gln Asp Val Val Val Leu Gly Ser Gln
245 250 255

Glu Gly Ala Met His Thr Ala Leu Thr Gly Ala Thr Glu Ile Gln Met
260 265 270

Ser Ser Gly Asn Leu Leu Phe Thr Gly His Leu Lys Cys Arg Leu Arg
275 280 285

Met Asp Lys Leu Gln Leu Lys Gly Met Ser Tyr Ser Met Cys Thr Gly
290 295 300

[0040]

Lys Phe Lys Val Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile
305 310 315 320

Val Ile Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro
325 330 335

Phe Glu Ile Met Asp Leu Glu Lys Arg His Val Leu Gly Arg Leu Ile
340 345 350

Thr Val Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu
355 360 365

Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro
370 375 380

Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln
385 390 395 400

Met Phe Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly
405 410 415

Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly Gly Val Phe Thr Ser Ile
420 425 430

Gly Lys Ala Leu His Gln Val Phe Gly Ala Ile Tyr Gly Ala Ala Phe
435 440 445

Ser Gly Val Ser Trp Thr Met Lys Ile Leu Ile Gly Val Ile Ile Thr
450 455 460

Trp Ile Gly Met Asn Ser Arg Ser Thr Ser Leu Ser Val Thr Leu Val
465 470 475 480

[0041]

Leu Val Gly Ile Val Thr Leu Tyr Leu Gly Val Met Val Gln Ala
485 490 495

<210> 14
<211> 495
<212> PRT
<213> 人共序列

<220>
<223> E BID/V585

<400> 14

Met Arg Cys Ile Gly Ile Ser Asn Arg Asp Phe Val Glu Gly Val Ser
1 5 10 15

Gly Gly Ser Trp Val Asp Ile Val Leu Glu His Gly Ser Cys Val Thr
20 25 30

Thr Met Ala Lys Asn Lys Pro Thr Leu Asp Phe Glu Leu Ile Lys Thr
35 40 45

Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys Tyr Cys Ile Glu Ala Lys
50 55 60

Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys Pro Thr Gln Gly Glu Pro
65 70 75 80

Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe Ile Cys Lys His Ser Met
85 90 95

Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Gly
100 105 110

[0042]

Ile Val Thr Cys Ala Met Phe Thr Cys Lys Lys Asn Met Glu Gly Lys
115 120 125

Val Val Gln Pro Glu Asn Leu Glu Tyr Thr Ile Val Ile Thr Pro His
130 135 140

Ser Gly Glu Glu His Ala Val Gly Asn Asp Thr Gly Lys His Gly Lys
145 150 155 160

Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile Thr Glu Ala Glu Leu Thr
165 170 175

Gly Tyr Gly Thr Val Thr Met Glu Cys Ser Pro Arg Thr Gly Leu Asp
180 185 190

Phe Asn Glu Met Val Leu Leu Gln Met Glu Asp Lys Ala Trp Leu Val
195 200 205

His Arg Gln Trp Phe Leu Asp Leu Pro Leu Pro Trp Leu Pro Gly Ala
210 215 220

Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys Glu Thr Leu Val Thr Phe
225 230 235 240

Lys Asn Pro His Ala Lys Lys Gln Asp Val Val Val Leu Gly Ser Gln
245 250 255

Glu Gly Ala Met His Thr Ala Leu Thr Gly Ala Thr Glu Ile Gln Met
260 265 270

Ser Ser Gly Asn Leu Leu Phe Thr Gly His Leu Lys Cys Arg Leu Arg
275 280 285

[0043]

Met Asp Lys Leu Gln Leu Lys Gly Met Ser Tyr Ser Met Cys Thr Gly
290 295 300

Lys Phe Lys Ile Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile
305 310 315 320

Val Ile Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro
325 330 335

Phe Glu Ile Met Asp Leu Glu Lys Arg His Val Leu Gly Arg Leu Ile
340 345 350

Thr Val Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu
355 360 365

Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro
370 375 380

Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln
385 390 395 400

Met Phe Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly
405 410 415

Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly Gly Val Phe Thr Ser Ile
420 425 430

Gly Lys Ala Leu His Gln Val Phe Gly Ala Ile Tyr Gly Ala Ala Phe
435 440 445

Ser Gly Val Ser Trp Thr Met Lys Ile Leu Ile Gly Val Ile Ile Thr
450 455 460

[0044]

Trp Ile Gly Met Asn Ser Arg Ser Thr Ser Leu Ser Val Ser Leu Val
465 470 475 480

Leu Val Gly Val Val Thr Leu Tyr Leu Gly Val Met Val Gln Ala
485 490 495

<210> 15
<211> 495
<212> PRT
<213> 人共序列

<220>
<223> E PR/DB023

<400> 15

Met Arg Cys Ile Gly Ile Ser Asn Arg Asp Phe Val Glu Gly Val Ser
1 5 10 15

Gly Gly Ser Trp Val Asp Ile Val Leu Glu His Gly Ser Cys Val Thr
20 25 30

Thr Met Ala Lys Asn Lys Pro Thr Leu Asp Phe Glu Leu Ile Lys Thr
35 40 45

Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys Tyr Cys Ile Glu Ala Lys
50 55 60

Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys Pro Thr Gln Gly Glu Pro
65 70 75 80

Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe Ile Cys Lys His Ser Met
85 90 95

Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Gly
100 105 110

[0045]

Ile Val Thr Cys Ala Met Phe Thr Cys Lys Lys Asn Met Glu Gly Lys
115 120 125

Val Val Leu Pro Glu Asn Leu Glu Tyr Thr Ile Val Ile Thr Pro His
130 135 140

Ser Gly Glu Glu His Ala Val Gly Asn Asp Thr Gly Lys His Gly Lys
145 150 155 160

Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile Thr Glu Ala Glu Leu Thr
165 170 175

Gly Tyr Gly Thr Val Thr Met Glu Cys Ser Pro Arg Thr Gly Leu Asp
180 185 190

Phe Asn Glu Met Val Leu Leu Gln Met Glu Asp Lys Ala Trp Leu Val
195 200 205

His Arg Gln Trp Phe Leu Asp Leu Pro Leu Pro Trp Leu Pro Gly Ala
210 215 220

Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys Glu Thr Leu Val Thr Phe
225 230 235 240

Lys Asn Pro His Ala Lys Lys Gln Asp Val Val Val Leu Gly Ser Gln
245 250 255

Glu Gly Ala Met His Thr Ala Leu Thr Gly Ala Thr Glu Ile Gln Met
260 265 270

Ser Ser Gly Asn Leu Leu Phe Thr Gly His Leu Lys Cys Arg Leu Arg
275 280 285

[0046]

Met Asp Lys Leu Gln Leu Lys Gly Met Ser Tyr Ser Met Cys Thr Gly
290 295 300

Lys Phe Lys Ile Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile
305 310 315 320

Val Ile Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro
325 330 335

Phe Glu Ile Met Asp Leu Glu Lys Arg His Val Leu Gly Arg Leu Ile
340 345 350

Thr Val Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu
355 360 365

Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro
370 375 380

Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln
385 390 395 400

Met Phe Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly
405 410 415

Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly Gly Val Phe Thr Ser Ile
420 425 430

Gly Lys Ala Leu His Gln Val Phe Gly Ala Ile Tyr Gly Ala Ala Phe
435 440 445

Ser Gly Val Ser Trp Thr Met Lys Ile Leu Ile Gly Val Ile Ile Thr
450 455 460

[0047]

Trp Ile Gly Met Asn Ser Arg Ser Thr Ser Leu Ser Val Ser Leu Val
465 470 475 480

Leu Val Gly Val Val Thr Leu Tyr Leu Gly Val Met Val Gln Ala
485 490 495

<210> 16
<211> 495
<212> PRT
<213> 人共序列

<220>
<223> E MD1280

<400> 16

Met Arg Cys Ile Gly Ile Ser Asn Arg Asp Phe Val Glu Gly Val Ser
1 5 10 15

Gly Gly Ser Trp Val Asp Ile Val Leu Glu His Gly Ser Cys Val Thr
20 25 30

Thr Met Ala Lys Asn Lys Pro Thr Leu Asp Phe Glu Leu Ile Lys Thr
35 40 45

Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys Tyr Cys Ile Glu Ala Lys
50 55 60

Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys Pro Thr Gln Gly Glu Pro
65 70 75 80

Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe Val Cys Lys His Ser Met
85 90 95

[0048]

Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Gly
100 105 110

Ile Val Thr Cys Ala Met Phe Thr Cys Lys Lys Asn Met Glu Gly Lys
115 120 125

Ile Val Gln Pro Glu Asn Leu Glu Tyr Thr Ile Val Ile Thr Pro His
130 135 140

Ser Gly Glu Glu His Ala Val Gly Asn Asp Thr Gly Lys His Gly Lys
145 150 155 160

Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile Thr Glu Ala Glu Leu Thr
165 170 175

Gly Tyr Gly Thr Val Thr Met Glu Cys Ser Pro Arg Thr Gly Leu Asp
180 185 190

Phe Asn Glu Met Val Leu Leu Gln Met Glu Asp Lys Ala Trp Leu Val
195 200 205

His Arg Gln Trp Phe Leu Asp Leu Pro Leu Pro Trp Leu Pro Gly Ala
210 215 220

Asp Thr Gln Gly Ser Asn Trp Ile Gln Lys Glu Thr Leu Val Thr Phe
225 230 235 240

Lys Asn Pro His Ala Lys Lys Gln Asp Val Val Val Leu Gly Ser Gln
245 250 255

Glu Gly Ala Met His Thr Ala Leu Thr Gly Ala Thr Glu Ile Gln Met
260 265 270

[0049]

Ser Ser Gly Asn Leu Leu Phe Thr Gly His Leu Lys Cys Arg Leu Arg
275 280 285

Met Asp Lys Leu Gln Leu Lys Gly Met Ser Tyr Ser Met Cys Thr Gly
290 295 300

Lys Phe Lys Ile Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile
305 310 315 320

Val Ile Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro
325 330 335

Phe Glu Ile Met Asp Leu Glu Lys Arg His Val Leu Gly Arg Leu Ile
340 345 350

Thr Val Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu
355 360 365

Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro
370 375 380

Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln
385 390 395 400

Met Phe Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly
405 410 415

Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly Gly Val Phe Thr Ser Ile
420 425 430

Gly Lys Ala Leu His Gln Val Phe Gly Ala Ile Tyr Gly Ala Ala Phe
435 440 445

[0050]

Ser Gly Val Ser Trp Thr Met Lys Ile Leu Ile Gly Val Ile Ile Thr
450 455 460

Trp Ile Gly Met Asn Ser Arg Ser Thr Ser Leu Ser Val Ser Leu Val
465 470 475 480

Leu Val Gly Ile Ile Thr Leu Tyr Leu Gly Ala Met Val Gln Ala
485 490 495

<210> 17
<211> 75
<212> PRT
<213> 人共序列

<220>
<223> M 共有序列

<400> 17

Ser Val Ala Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr
1 5 10 15

Glu Thr Trp Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile
20 25 30

Glu Thr Trp Ile Leu Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile
35 40 45

Leu Ala Tyr Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe
50 55 60

Ile Leu Leu Thr Ala Val Ala Pro Ser Met Thr
65 70 75

<210> 18

[0051]

<211> 495
<212> PRT
<213> 人共序列

<220>
<223> E 传播毒株

<400> 18

Met Arg Cys Ile Gly Ile Ser Asn Arg Asp Phe Val Glu Gly Val Ser
1 5 10 15

Gly Gly Ser Trp Val Asp Ile Val Leu Glu His Gly Ser Cys Val Thr
20 25 30

Thr Met Ala Lys Asn Lys Pro Thr Leu Asp Phe Glu Leu Ile Lys Thr
35 40 45

Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys Tyr Cys Ile Glu Ala Lys
50 55 60

Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys Pro Thr Gln Gly Glu Pro
65 70 75 80

Ser Leu Lys Glu Glu Gln Asp Lys Arg Phe Val Cys Lys His Ser Met
85 90 95

Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Gly
100 105 110

Ile Val Thr Cys Ala Met Phe Thr Cys Lys Lys Asn Met Glu Gly Lys
115 120 125

Ile Val Gln Pro Glu Asn Leu Glu Tyr Thr Ile Val Val Thr Pro His
130 135 140

[0052]

Ser Gly Glu Glu His Ala Val Gly Asn Asp Thr Gly Lys His Gly Lys
145 150 155 160

Glu Ile Lys Val Thr Pro Gln Ser Ser Ile Thr Glu Ala Glu Leu Thr
165 170 175

Gly Tyr Gly Thr Val Thr Met Glu Cys Ser Pro Arg Thr Gly Leu Asp
180 185 190

Phe Asn Glu Met Val Leu Leu Gln Met Glu Asn Lys Ala Trp Leu Val
195 200 205

His Arg Gln Trp Phe Leu Asp Leu Pro Leu Pro Trp Leu Pro Gly Ala
210 215 220

Asp Lys Gln Glu Ser Asn Trp Ile Gln Lys Glu Thr Leu Val Thr Phe
225 230 235 240

Lys Asn Pro His Ala Lys Lys Gln Asp Val Val Val Leu Gly Ser Gln
245 250 255

Glu Gly Ala Met His Thr Ala Leu Thr Gly Ala Thr Glu Ile Gln Met
260 265 270

Ser Ser Gly Asn Leu Leu Phe Thr Gly His Leu Lys Cys Arg Leu Arg
275 280 285

Met Asp Lys Leu Gln Leu Lys Gly Met Ser Tyr Ser Met Cys Thr Gly
290 295 300

Lys Phe Lys Val Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile
305 310 315 320

[0053]

Val Ile Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro
325 330 335

Phe Glu Ile Met Asp Leu Glu Lys Arg Tyr Val Leu Gly Arg Leu Ile
340 345 350

Thr Val Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu
355 360 365

Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro
370 375 380

Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln
385 390 395 400

Met Phe Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly
405 410 415

Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly Gly Val Phe Thr Ser Ile
420 425 430

Gly Lys Ala Leu His Gln Val Phe Gly Ala Ile Tyr Gly Ala Ala Phe
435 440 445

Ser Gly Val Ser Trp Thr Met Lys Ile Leu Ile Gly Val Ile Ile Thr
450 455 460

Trp Ile Gly Met Asn Ser Arg Ser Thr Ser Leu Ser Val Ser Leu Val
465 470 475 480

Leu Val Gly Ile Val Thr Leu Tyr Leu Gly Val Met Val Gln Ala
485 490 495

[0054]

<210> 19
<211> 75
<212> PRT
<213> 人共序列

<220>
<223> M LAV2

<400> 19

Ser Val Ala Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr
1 5 10 15

Glu Thr Trp Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile
20 25 30

Glu Thr Trp Ile Leu Arg His Pro Gly Phe Thr Met Met Ala Ala Ile
35 40 45

Leu Ala Tyr Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe
50 55 60

Ile Leu Leu Thr Ala Val Thr Pro Ser Met Thr
65 70 75

<210> 20
<211> 75
<212> PRT
<213> 人共序列

<220>
<223> M BID/V585

<400> 20

Ser Val Ala Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr
1 5 10 15

[0055]

Glu Thr Trp Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile
20 25 30

Glu Thr Trp Ile Leu Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile
35 40 45

Leu Ala Tyr Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe
50 55 60

Ile Leu Leu Thr Ala Val Ala Pro Ser Met Thr
65 70 75

<210> 21
<211> 75
<212> PRT
<213> 人共序列

<220>
<223> M PR/DB023

<400> 21

Ser Val Ala Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr
1 5 10 15

Glu Thr Trp Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile
20 25 30

Glu Thr Trp Ile Leu Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile
35 40 45

Leu Ala Tyr Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe
50 55 60

Ile Leu Leu Thr Ala Val Ala Pro Ser Met Thr

[0056]

65 70 75

<210> 22
<211> 75
<212> PRT
<213> 人共序列

<220>
<223> M MD1280

<400> 22

Ser Val Ala Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr
1 5 10 15

Glu Thr Trp Met Ser Ser Glu Gly Ala Trp Lys His Ala Gln Arg Ile
20 25 30

Glu Thr Trp Ile Leu Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile
35 40 45

Leu Ala Tyr Thr Val Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe
50 55 60

Ile Leu Leu Ala Ala Val Ala Pro Ser Met Thr
65 70 75

<210> 23
<211> 75
<212> PRT
<213> 人共序列

<220>
<223> M 临床试验传播毒株

<400> 23

Ser Val Ala Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr

[0057]

1 5 10 15

Glu Thr Trp Met Ser Ser Glu Gly Ala Trp Lys His Ala Gln Arg Ile
20 25 30

Glu Thr Trp Ile Leu Arg His Pro Gly Phe Thr Ile Met Ala Ala Ile
35 40 45

Leu Ala Tyr Thr Ile Gly Thr Thr His Phe Gln Arg Val Leu Ile Phe
50 55 60

Ile Leu Leu Thr Ala Val Ala Pro Ser Met Thr
65 70 75

<210> 24
<211> 10723
<212> RNA
<213> 人共序列

<220>
<223> VDV2

<400> 24
aguuguuagu cuacguggac cgacaaagac agauucuuug agggagcuaa gcucaaugua 60

guucuaacag uuuuuuaauu agagagcaga ucucugauga auaaccaacg gaaaaaggcg 120

aaaaacacgc cuuucaauau gcugaaacgc gagagaaacc gcgugucgac ugugcaacag 180

cugacaaaga gauucucacu uggaaugcug cagggacgag gaccauuaaa acuguucaug 240

gcccuggugg cguuccuucg uuuccuaaca aucccaccaa cagcagggau auugaagaga 300

uggggaacaa uuaaaaaauc aaaagcuauu aauguuuuga gaggguucag gaaagagauu 360

ggaaggaugc ugaacaucuu gaauaggaga cgcagaucug caggcaugau cauuaugcug 420

auuccaacag ugauggcguu ccauuuaacc acacguaacg gagaaccaca caugaucguc 480

[0058]

agcagacaag agaaagggaa aagucuucug uuuaaaacag agguuggcgu gaacaugugu 540

acccucaugg ccauggaccu uggugaauug ugugaagaca caaucacgua caagugcccc 600

cuucucaggc agaaugagcc agaagacaua gacuguuggu gcaacucuac guccacgugg 660

guaacuuaug ggacguguac caccauggga gaacauagaa gagaaaaaag aucaguggca 720

cucguuccac augugcgaau gggacuggag acacgaacug aaacauggau gucaucagaa 780

ggggccugga aacaugucca gagaauugaa acuuggaucu ugagacaucc aggcuucacc 840

augauggcag caauccuggc auacaccaua ggaacgacac auuuccaaag agcccugauu 900

uucaucuuac ugacagcugu cacuccuuca augacaaugc guugcauagg aaugucaaau 960

agagacuuug uggaaggggu uucaggagga agcuggguug acauagucuu agaacaugga 1020

agcuguguga cgacgauggc aaaaaacaaa ccaacauugg auuuugaacu gauaaaaaca 1080

gaagccaaac agccugccac ccuaaggaag uacuguauag aggcaaagcu aaccaacaca 1140

acaacagaau cucgcugccc aacacaaggg gaacccagcc uaaaugaaga gcaggacaaa 1200

agguucgucu gcaaacacuc caugguagac agaggauggg gaaauggaug uggacuauuu 1260

ggaaagggag gcauugugac cugugcuaug uucagaugca aaaagaacau ggaaggaaaa 1320

guugugcaac cagaaaacuu ggaauacacc auugugauaa caccucacuc aggggaagag 1380

caugcagucg gaaaugacac aggaaaacau ggcaaggaaa ucaaaauaac accacagagu 1440

uccaucacag aagcagaauu gacagguuau ggcacuguca caauggagug cucuccaaga 1500

acgggccucg acuucaauga gaugguguug cugcagaugg aaaauaaagc uuggcuggug 1560

cacaggcaau gguuccuaga ccugccguua ccaugguugc ccggagcgga cacacaagag 1620

ucaaauugga uacagaagga gacauugguc acuuucaaaa aucccccaugc gaagaaacag 1680

gauguuguug uuuuaggauc ccaagaaggg gccaugcaca cagcacuuac aggggccaca 1740

gaaauccaaa ugucaucagg aaacuuacuc uucacaggac aucucaagug caggcugaga 1800

[0059]

auggacaagc uacagcucaa aggaauguca uacucuaugu gcacaggaaa guuuaaaguu 1860

gugaaggaaa uagcagaaac acaacaugga acaauaguua ucagagugca auaugaaggg 1920

gacggcucuc caugcaagau cccuuuugag auaauggauu uggaaaaaag acaugucuua 1980

ggucgccuga uuacagucaa cccaauugug acagaaaaag auagcccagu caacauagaa 2040

gcagaaccuc cauuuggaga cagcuacauc aucauaggag uagagccggg acaacugaag 2100

cucaacuggu uuaagaaagg aaguucuauc ggccaaaugu uugagacaac aaugaggggg 2160

gcgaagagaa uggccauuuu aggugacaca gccugggauu uuggauccuu gggaggagug 2220

uuuacaucua uaggaaaggc ucuccaccaa gucuuuggag caaucuaugg agcugccuuc 2280

agugggguuu cauggacuau gaaaauccuc auaggaguca uuaucacaug gauaggaaug 2340

aauucacgca gcaccucacu gucugugaca cuaguauugg ugggaauugu gacacuguau 2400

uugggaguca uggugcaggc cgauaguggu ugcguuguga gcuggaaaaa caaagaacug 2460

aaauguggca gugggauuuu caucacagac aacgugcaca cauggacaga acaauacaaa 2520

uuccaaccag aauccccuuc aaaacuagcu ucagcuaucc agaaagccca ugaagaggac 2580

auuuguggaa uccgcucagu aacaagacug gagaaucuga uguggaaaca aauaacacca 2640

gaauugaauc acauucuauc agaaaaugag gugaaguuaa cuauuaugac aggagacauc 2700

aaaggaauca ugcaggcagg aaaacgaucu cugcggccuc agcccacuga gcugaaguau 2760

ucauggaaaa cauggggcaa agcaaaaaug cucucuacag agucucauaa ccagaccuuu 2820

cucauugaug gccccgaaac agcagaaugc cccaacacaa auagagcuug gaauucguug 2880

gaaguugaag acuauggcuu uggaguauuc accaccaaua uauggcuaaa auugaaagaa 2940

aaacaggaug uauucugcga cucaaaacuc augucagcgg ccauaaaaga caacagagcc 3000

guccaugccg auauggguua uuggauagaa agugcacuca augacacaug gaagauagag 3060

aaagccucuu ucauugaagu uaaaaacugc cacuggccaa aaucacacac ccucuggagc 3120

[0060]

aauggagugc uagaaaguga gaugauaauu ccaaagaauc ucgcuggacc agugucucaa 3180

cacaacuaua gaccaggcua ccauacacaa auaacaggac cauggcaucu agguaagcuu 3240

gagauggacu uugauuucug ugauggaaca acagugguag ugacugagga cugcggaaau 3300

agaggacccu cuuugagaac aaccacugcc ucuggaaaac ucauaacaga auggugcugc 3360

cgaucuugca cauuaccacc gcuaagauac agaggugagg augggugcug guacgggaug 3420

gaaaucagac cauugaagga gaaagaagag aauuugguca acuccuuggu cacagcugga 3480

caugggcagg ucgacaacuu uucacuagga gucuugggaa uggcauuguu ccuggaggaa 3540

augcuuagga cccgaguagg aacgaaacau gcaauacuac uaguugcagu uucuuuugug 3600

acauugauca cagggaacau guccuuuaga gaccugggaa gagugauggu uaugguaggc 3660

gccacuauga cggaugacau agguaugggc gugacuuauc uugcccuacu agcagccuuc 3720

aaagucagac caacuuuugc agcuggacua cucuugagaa agcugaccuc caaggaauug 3780

augaugacua cuauaggaau uguacuccuc ucccagagca ccauaccaga gaccauucuu 3840

gaguugacug augcguuagc cuuaggcaug augguccuca aaauggugag aaauauggaa 3900

aaguaucaau uggcagugac uaucauggcu aucuugugcg ucccaaacgc agugauauua 3960

caaaacgcau ggaaagugag uugcacaaua uuggcagugg uguccguuuc cccacuguuc 4020

uuaacauccu cacagcaaaa aacagauugg auaccauuag cauugacgau caaaggucuc 4080

aauccaacag cuauuuuucu aacaacccuc ucaagaacca gcaagaaaag gagcuggcca 4140

uuaaaugagg cuaucauggc agucgggaug gugagcauuu uagccaguuc ucuccuaaaa 4200

aaugauauuc ccaugacagg accauuagug gcuggagggc uccucacugu gugcuacgug 4260

cucacuggac gaucggccga uuuggaacug gagagagcag ccgaugucaa augggaagac 4320

caggcagaga uaucaggaag caguccaauc cugucaauaa caauaucaga agaugguagc 4380

augucgauaa aaaaugaaga ggaagaacaa acacugacca uacucauuag aacaggauug 4440

[0061]

cuggugaucu caggacuuuu uccuguauca auaccaauca cggcagcagc augguaccug 4500

ugggaaguga agaaacaacg ggccggagua uuguggggaug uuccuucacc cccacccaug 4560

ggaaaggcug aacuggaaga uggagccuau agaauuaagc aaaaagggau ucuuggauau 4620

ucccagaucg gagccggagu uuacaaagaa ggaacauucc auacaaugug gcaugucaca 4680

cguggcgcug uucuaaugca uaaaggaaag aggauugaac caacaugggc ggacgucaag 4740

aaagaccuaa uaucauaugg aggaggcugg aaguuagaag gagaauggaa ggaaggagaa 4800

gaaguccagg uauuggcacu ggagccugga aaaaauccaa gagccgucca aacgaaaccu 4860

ggucuuuuca aaaccaacgc cggaacaaua ggugcuguau cucuggacuu uucuccugga 4920

acgucaggau cuccaauuau cgacaaaaaa ggaaaaguug ugggucuuua ugguaauggu 4980

guuguuacaa ggaguggagc auaugugagu gcuauagccc agacugaaaa aagcauugaa 5040

gacaacccag agaucgaaga ucacauuuuc cgaaagagaa gacugaccau cauggaccuc 5100

cacccaggag cgggaaagac gaagagauac cuuccggcca uagucagaga agcuauaaaa 5160

cgggguuuga gaacauuaau cuuggccccc acuagaguug uggcagcuga aauggaggaa 5220

gcccuuagag gacuuccaau aagauaccag accccagcca ucagagcuga gcacaccggg 5280

cgggagauug uggaccuaau gugucaugcc acauuuacca ugaggcugcu aucaccaguu 5340

agagugccaa acuacaaccu gauuaucaug gacgaagccc auuucacaga cccagcaagu 5400

auagcagcua gaggauacau cucaacucga guggagaugg gugaggcagc ugggauuuuu 5460

augacagcca cucccccggg aagcagagac ccauuuccuc agagcaaugc accaaucaua 5520

gaugaagaaa gagaaauccc ugaacgcucg uggaauuccg gacaugaaug ggucacggau 5580

uuuaaaggga agacuguuug guucguucca aguauaaaag caggaaauga uauagcagcu 5640

ugccugagga aaaauggaaa gaaagugaua caacucagua ggaagaccuu ugauucugag 5700

uaugucaaga cuagaaccaa ugauugggac uucgugguua caacugacau uucagaaaug 5760

[0062]

ggugccaauu ucaaggcuga gaggguuaua gaccccagac gcugcaugaa accagucaua 5820

cuaacagaug gugaagagcg ggugauucug gcaggaccua ugccagugac ccacucuagu 5880

gcagcacaaa gaagagggag aauaggaaga aauccaaaaa augagaauga ccaguacaua 5940

uacaugggg aaccucugga aaaugaugaa gacugugcac acuggaaaga agcuaaaaug 6000

cuccuagaua acaucaacac gccagaagga aucauuccua gcauguucga accagagcgu 6060

gaaaaggugg augccauuga uggcgaauac cgcuugagag gagaagcaag gaaaaccuuu 6120

guagacuuaa ugagaagagg agaccuacca gucugguugg ccuacagagu ggcagcugaa 6180

ggcaucaacu acgcagacag aagguggugu uuugauggag ucaagaacaa ccaaauccua 6240

gaagaaaacg uggaaguuga aaucuggaca aaagaagggg aaaggaagaa auugaaaccc 6300

agaugguugg augcuaggau cuauucugac ccacuggcgc uaaaagaauu uaaggaauuu 6360

gcagccggaa gaaagucucu gacccugaac cuaaucacag aaauggguag gcucccaacc 6420

uucaugacuc agaaggcaag agacgcacug gacaacuuag cagugcugca cacggcugag 6480

gcagguggaa gggcguacaa ccaugcucuc agugaacugc cggagacccu ggagacauug 6540

cuuuuacuga cacuucuggc uacagucacg ggagggaucu uuuuauucuu gaugagcgca 6600

aggggcauag ggaagaugac ccugggaaug ugcugcauaa ucacggcuag cauccuccua 6660

ugguacgcac aaauacagcc acacuggaua gcagcuucaa uaauacugga guuuuuucuc 6720

auaguuuugc uuauuccaga accugaaaaa cagagaacac cccaagacaa ccaacugacc 6780

uacguuguca uagccauccu cacaguggug gccgcaacca uggcaaacga gauggguuuc 6840

cuagaaaaaa cgaagaaaga ucucggauug ggaagcauug caacccagca acccgagagc 6900

aacauccugg acauagaucu acguccugca ucagcaugga cgcuguaugc cguggccaca 6960

acauuuguua caccaauguu gagacauagc auugaaaauu ccucagugaa uguguccuua 7020

acagcuauag ccaaccaagc cacaguguua augggucucg ggaaaggaug gccauuguca 7080

[0063]

aagauggaca ucggaguucc ccuucucgcc auuggaugcu acucacaagu caaccccaua 7140

acucucacag cagcucuuuu cuuauuggua gcacauuaug ccaucauagg gccaggacuc 7200

caagcaaaag caaccagaga agcucagaaa agagcagcgg cgggcaucau gaaaaaccca 7260

acugucgaug gaauaacagu gauugaccua gauccaauac cuuaugaucc aaaguuugaa 7320

aagcaguugg gacaaguaau gcuccuaguc cucugcguga cucaaguauu gaugaugagg 7380

acuacauggg cucuguguga ggcuuuaacc uuagcuaccg ggcccaucuc cacauugugg 7440

gaaggaaauc cagggagguu uuggaacacu accauugcgg ugucaauggc uaacauuuuu 7500

agagggaguu acuuggccgg agcuggacuu cucuuuucua uuaugaagaa cacaaccaac 7560

acaagaaggg gaacuggcaa cauaggagag acgcuuggag agaaauggaa aagccgauug 7620

aacgcauugg gaaaaaguga auuccagauc uacaagaaaa guggaaucca ggaaguggau 7680

agaaccuuag caaaagaagg cauuaaaaga ggagaaacgg accaucacgc ugugucgcga 7740

ggcucagcaa aacugagaug guucguugag agaaacaugg ucacaccaga agggaaagua 7800

guggaccucg guuguggcag aggaggcugg ucauacuauu guggaggacu aaagaaugua 7860

agagaaguca aaggccuaac aaaaggagga ccaggacacg aagaacccau ccccauguca 7920

acauaugggu ggaaucuagu gcgucuucaa aguggaguug acguuuucuu caucccgcca 7980

gaaaagugug acacauuauu gugugacaua ggggagucau caccaaaucc cacaguggaa 8040

gcaggacgaa cacucagagu ccuuaacuua guagaaaauu gguugaacaa caacacucaa 8100

uuuugcauaa agguucucaa cccauauaug cccucaguca uagaaaaaau ggaagcacua 8160

caaaggaaau auggaggagc cuuagugagg aauccacucu cacgaaacuc cacacaugag 8220

auguacuggg uauccaaugc uuccgggaac auagugucau cagugaacau gauuucaagg 8280

auguugauca acagauuuac aaugagauac aagaaagcca cuuacgagcc ggauguugac 8340

cucggaagcg gaacccguaa caucgggauu gaaagugaga uaccaaaccu agauauaauu 8400

[0064]

gggaaaagaa uagaaaaaau aaagcaagag caugaaacau cauggcacua ugaccaagac 8460

cacccauaca aaacgugggc auaccauggu agcuaugaaa caaaacagac uggaucagca 8520

ucauccaugg ucaacggagu ggucaggcug cugacaaaac cuugggacgu ugucccaug 8580

gugacacaga uggcaaugac agacacgacu ccauuuggac aacagcgcgu uuuuaaagag 8640

aaaguggaca cgagaaccca agaaccgaaa gaaggcacga agaaacuaau gaaaauaaca 8700

gcagaguggc uuuggaaaga auuagggaag aaaaagacac ccaggaugug caccagagaa 8760

gaauucacaa gaaaggugag aagcaaugca gccuuggggg ccauauucac ugaugagaac 8820

aaguggaagu cggcacguga ggcuguugaa gauaguaggu uuugggagcu gguugacaag 8880

gaaaggaauc uccaucuuga aggaaagugu gaaacaugug uguacaacau gaugggaaaa 8940

agagagaaga agcuagggga auucggcaag gcaaaaggca gcagagccau augguacaug 9000

uggcuuggag cacgcuucuu agaguuugaa gcccuaggau ucuuaaauga agaucacugg 9060

uucuccagag agaacucccu gaguggagug gaaggagaag ggcugcacaa gcuagguuac 9120

auucuaagag acgugagcaa gaaagaggga ggagcaaugu augccgauga caccgcagga 9180

ugggauacaa aaaucacacu agaagaccua aaaaaugaag agaugguaac aaaccacaug 9240

gaaggagaac acaagaaacu agccgaggcc auuuucaaac uaacguacca aaacaaggug 9300

gugcgugugc aaagaccaac accaagaggc acaguaaugg acaucauauc gagaagagac 9360

caaagaggua guggacaagu uggcaccuau ggacucaaua cuuucaccaa uauggaagcc 9420

caacuaauca gacagaugga gggagaagga gucuuuaaaa gcauucagca ccuaacaauc 9480

acagaagaaa ucgcugugca aaacugguua gcaagagugg ggcgcgaaag guuaucaaga 9540

auggccauca guggagauga uuguguugug aaaccuuuag augacagguu cgcaagcgcu 9600

uuaacagcuc uaaaugacau gggaaagauu aggaaagaca uacaacaaug ggaaccuuca 9660

agaggaugga augauuggac acaagugccc uucuguucac accauuucca ugaguuaauc 9720

[0065]

augaaagacg gucgcguacu cguuguucca uguagaaacc aagaugaacu gauuggcaga 9780

gcccgaaucu cccaaggagc aggguggucu uugcgggaga cggccuguuu ggggaagucu 9840

uacgcccaaa uguggagcuu gauguacuuc cacagacgcg accucaggcu ggcggcaaau 9900

gcuauuugcu cggcaguacc aucacauugg guuccaacaa gucgaacaac cugguccaua 9960

caugcuaaac augaauggau gacaacggaa gacaugcuga cagucuggaa cagggugugg 10020

auucaagaaa acccauggau ggaagacaaa acuccagugg aaacauggga ggaaauccca 10080

uacuugggga aaagagaaga ccaauggugc ggcucauuga uuggguuaac aagcagggcc 10140

accugggcaa agaacaucca agcagcaaua aaucaaguua gaucccuuau aggcaaugaa 10200

gaauacacag auuacaugcc auccaugaaa agauucagaa gagaagagga agaagcagga 10260

guucuguggu agaaagcaaa acuaacauga aacaaggcua gaagucaggu cggauuaagc 10320

cauaguacgg aaaaaacuau gcuaccugug agccccgucc aaggacguua aaagaaguca 10380

ggccaucaua aaugccauag cuugaguaaa cuaugcagcc uguagcucca ccugagaagg 10440

uguaaaaaau ccgggaggcc acaaaccaug gaagcuguac gcauggcgua guggacuagc 10500

gguuagggga gaccccuccc uuacaaaucg cagcaacaau gggggcccaa ggcgagauga 10560

agcuguaguc ucgcuggaag gacuagaggu uagaggagac ccccccgaaa caaaaaacag 10620

cauauugacg cugggaaaga ccagagaucc ugcugucucc ucagcaucau uccaggcaca 10680

gaacgccaga aaauggaaug gugcuguuga aucaacaggu ucu 10723

<210> 25
<211> 1983
<212> RNA
<213> 人共序列

<220>
<223> prM+E VDV2

<400> 25

[0066]

uuccauuuaa ccacacguaa cggagaacca cacaugaucg ucagcagaca agagaaaggg 60

aaaagucuuc uguuuaaaac agagguuggc gugaacaugu guacccucau ggccauggac 120

cuuggugaau ugugugaaga cacaaucacg uacaaguguc cccuucucag gcagaaugag 180

ccagaagaca uagacuguug gugcaacucu acguccacgu ggguaacuua ugggacgugu 240

accaccaugg gagaacauag aagagaaaaa agaucagugg cacucguucc acaugugcga 300

augggacugg agacacgaac ugaaacaugg augucaucag aaggggccug gaaacauguc 360

cagagaauug aaacuuggau cuugagacau ccaggcuuca ccaugauggc agcaauccug 420

gcauacacca uaggaacgac acauuuccaa agagcccuga uuuucaucuu acugacagcu 480

gucacuccuu caaugacaau gcguugcaua ggaaugucaa auagagacuu uguggaaggg 540

guuucaggag gaagcugggu ugacauaguc uuagaacaug gaagcugugu gacgacgaug 600

gcaaaaaaca aaccaacauu ggauuuugaa cugauaaaaa cagaagccaa acagccugcc 660

acccuaagga aguacuguau agaggcaaag cuaaccaaca caacaacaga aucucgcugc 720

ccaacacaag gggaacccag ccuaaaugaa gagcaggaca aaagguucgu cugcaaacac 780

uccaugguag acagaggaug gggaaaugga uguggacuau uuggaaaggg aggcauugug 840

accugugcua uguucagaug caaaaagaac auggaaggaa aaguugugca accagaaaac 900

uuggaauaca ccauugugau aacaccucac ucaggggaag agcaugcagu cggaaaugac 960

acaggaaaac auggcaagga aaucaaaaua acaccacaga guuccaucac agaagcagaa 1020

uugacagguu auggcacugu cacaauggag ugcucuccaa gaacgggccu cgacuucaau 1080

gagauggugu ugcugcagau ggaaaauaaa gcuuggcugg ugcacaggca augguuccua 1140

gaccugccgu uaccaugguu gcccggagcg gacacacaag agucaaauug gauacagaag 1200

gagacauugg ucacuuucaa aaauccccau gcgaagaaac aggauguugu uguuuuagga 1260

ucccaagaag gggccaugca cacagcacuu acaggggcca cagaaaucca aaugucauca 1320

[0067]

ggaaacuuac ucuucacagg acaucucaag ugcaggcuga gaauggacaa gcuacagcuc 1380

aaaggaaugu cauacucuau gugcacagga aaguuuaaag uugugaagga aauagcagaa 1440

acacaacaug gaacaauagu uaucagagug caauaugaag gggacggcuc uccaugcaag 1500

aucccuuuug agauaaugga uuuggaaaaa agacaugucu uaggucgccu gauuacaguc 1560

aacccaauug ugacagaaaa agauagccca gucaacauag aagcagaacc uccauuugga 1620

gacagcuaca ucaucauagg aguagagccg ggacaacuga agcucaacug guuuaagaaa 1680

ggaaguucua ucggccaaau guuugagaca acaaugaggg gggcgaagag aauggccauu 1740

uuaggugaca cagccuggga uuuuggaucc uugggaggag uguuuacauc uauaggaaag 1800

gcucuccacc aagucuuugg agcaaucuau ggagcugccu ucagugggguu uucauggacu 1860

augaaaaucc ucauaggagu cauuaucaca uggauaggaa ugaauucacg cagcaccuca 1920

cugucuguga cacuaguauu ggugggaauu gugacacugu auuugggagu cauggugcag 1980

gcc 1983

<210> 26
<211> 495
<212> PRT
<213> 人共序列

<220>
<223> E VDV2

<400> 26

Met Arg Cys Ile Gly Met Ser Asn Arg Asp Phe Val Glu Gly Val Ser
1 5 10 15

Gly Gly Ser Trp Val Asp Ile Val Leu Glu His Gly Ser Cys Val Thr
20 25 30

Thr Met Ala Lys Asn Lys Pro Thr Leu Asp Phe Glu Leu Ile Lys Thr

[0068]

35 40 45

Glu Ala Lys Gln Pro Ala Thr Leu Arg Lys Tyr Cys Ile Glu Ala Lys
50 55 60

Leu Thr Asn Thr Thr Thr Glu Ser Arg Cys Pro Thr Gln Gly Glu Pro
65 70 75 80

Ser Leu Asn Glu Glu Gln Asp Lys Arg Phe Val Cys Lys His Ser Met
85 90 95

Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Gly
100 105 110

Ile Val Thr Cys Ala Met Phe Arg Cys Lys Lys Asn Met Glu Gly Lys
115 120 125

Val Val Gln Pro Glu Asn Leu Glu Tyr Thr Ile Val Ile Thr Pro His
130 135 140

Ser Gly Glu Glu His Ala Val Gly Asn Asp Thr Gly Lys His Gly Lys
145 150 155 160

Glu Ile Lys Ile Thr Pro Gln Ser Ser Ile Thr Glu Ala Glu Leu Thr
165 170 175

Gly Tyr Gly Thr Val Thr Met Glu Cys Ser Pro Arg Thr Gly Leu Asp
180 185 190

Phe Asn Glu Met Val Leu Leu Gln Met Glu Asn Lys Ala Trp Leu Val
195 200 205

His Arg Gln Trp Phe Leu Asp Leu Pro Leu Pro Trp Leu Pro Gly Ala

[0069]

210 215 220

Asp Thr Gln Glu Ser Asn Trp Ile Gln Lys Glu Thr Leu Val Thr Phe
225 230 235 240

Lys Asn Pro His Ala Lys Lys Gln Asp Val Val Val Leu Gly Ser Gln
245 250 255

Glu Gly Ala Met His Thr Ala Leu Thr Gly Ala Thr Glu Ile Gln Met
260 265 270

Ser Ser Gly Asn Leu Leu Phe Thr Gly His Leu Lys Cys Arg Leu Arg
275 280 285

Met Asp Lys Leu Gln Leu Lys Gly Met Ser Tyr Ser Met Cys Thr Gly
290 295 300

Lys Phe Lys Val Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile
305 310 315 320

Val Ile Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro
325 330 335

Phe Glu Ile Met Asp Leu Glu Lys Arg His Val Leu Gly Arg Leu Ile
340 345 350

Thr Val Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu
355 360 365

Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro
370 375 380

Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln

[0070]

385 390 395 400

Met Phe Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly
405 410 415

Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly Gly Val Phe Thr Ser Ile
420 425 430

Gly Lys Ala Leu His Gln Val Phe Gly Ala Ile Tyr Gly Ala Ala Phe
435 440 445

Ser Gly Val Ser Trp Thr Met Lys Ile Leu Ile Gly Val Ile Ile Thr
450 455 460

Trp Ile Gly Met Asn Ser Arg Ser Thr Ser Leu Ser Val Thr Leu Val
465 470 475 480

Leu Val Gly Ile Val Thr Leu Tyr Leu Gly Val Met Val Gln Ala
485 490 495

<210> 27
<211> 75
<212> PRT
<213> 人共序列

<220>
<223> M VDV2

<400> 27

Ser Val Ala Leu Val Pro His Val Arg Met Gly Leu Glu Thr Arg Thr
1 5 10 15

Glu Thr Trp Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile
20 25 30

[0071]

Glu Thr Trp Ile Leu Arg His Pro Gly Phe Thr Met Met Ala Ala Ile
35 40 45

Leu Ala Tyr Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe
50 55 60

Ile Leu Leu Thr Ala Val Thr Pro Ser Met Thr
65 70 75

图 1: YF-VAX cDNA 通过 RT-PCT 的构建和克隆

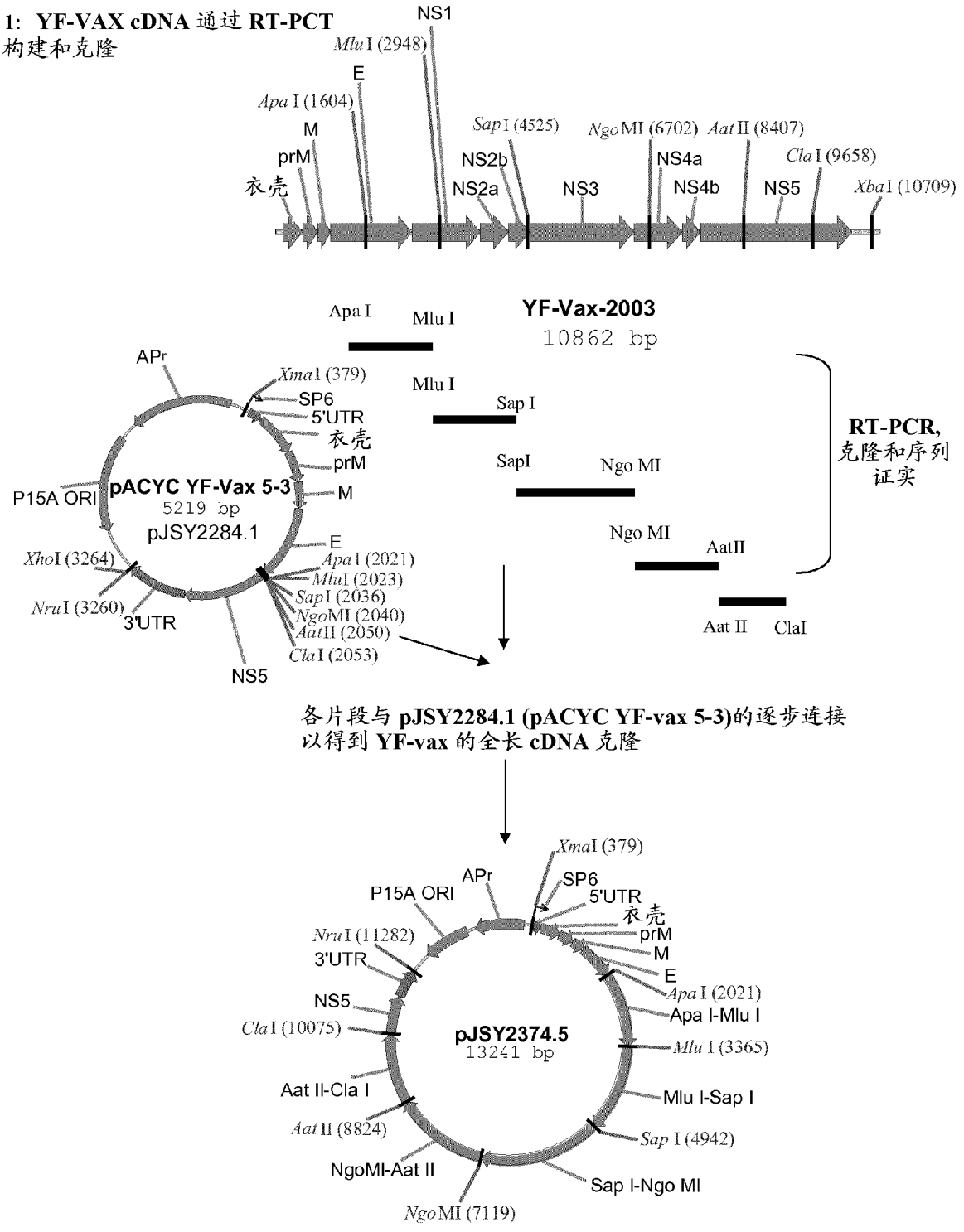


图 1