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(54) Title: COMPOSITIONS FOR BOOSTER VACCINATION AGAINST DENGU

(57) Abstract: The present invention is directed to a method of booster vaccination and to a vaccine composition for use in such a method, for inducing in a human subject a neutralizing antibody response, wherein said subject has previously received a primary vaccination against each of serotypes 1 to 4 of dengue virus and was dengue naïve before said primary vaccination, said composition comprising a dengue antigen of at least one of serotypes 1 to 4 or a nucleic acid construct capable of expressing said antigens in the subject, wherein said booster vaccination results in a 2-fold increase in the neutralizing antibody titre against each of serotypes 1 to 4. The invention is also directed to a method of inducing in a human subject a neutralizing antibody response comprising the administration of a vaccine composition, or to a vaccine composition for use in such a method, said composition comprising a dengue antigen of each of serotypes 1 to 4, or a nucleic acid construct capable of expressing in said subject a dengue antigen of each of serotypes 1 to 4; wherein said composition is administered as a primary vaccination, followed by a booster vaccination, and wherein the human subject is initially dengue naïve.

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COMPOSITIONS FOR BOOSTER VACCINATION AGAINST DENGUE

This application incorporates-by-reference nucleotide and/or amino acid sequences which are present in the accompanying sequence listing as part of this application.

FIELD OF THE INVENTION

The present invention relates to vaccine compositions and uses of such compositions as booster vaccines in a method of generating a neutralizing antibody response against dengue virus, in human subjects.

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 390 million cases of dengue every year and roughly 96 million people have clinically apparent disease. Each year, an estimated 500,000 people, including children, have a severe form of dengue requiring hospitalization, which puts a huge strain on health care systems during outbreaks. Approximately 2.5% of those affected with a severe form of dengue will die (World Health Organization. Dengue and dengue haemorrhagic fever, Fact sheet N°117, Updated May 2015. Available from URL: http://www.who.int/mediacentre/factsheets/fs117/en/.) Thus, according to WHO, there is an urgent need to develop a safe and effective vaccine against the four serotypes of dengue virus to protect people in endemic countries.

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 viruses are positive-sense, single-stranded RNA viruses.

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 haemorrhages, 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.

Severe forms of dengue disease including dengue haemorrhagic fever (DHF) are 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 viraemia (the severity of the disease being associated with the level of viraemia; 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 disease infections are endemic in more than 100 tropical countries and DHF has been documented in 60 of these countries (Gubler, 2002, TRENDS in Microbiology, 10: 100-103).

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). Incidence of dengue disease has increased in older age groups in many countries where dengue is endemic (Sabchareon *et al*, 2012, Lancet, 380, 1559-1567; Messina *et al.*, 2014, Trends Microbiol., 22, 138-146).

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. It is 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 virus.

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.

The Applicant has previously developed a dengue vaccine, marketed under the commercial name Dengvaxia®. The vaccine efficacy of this dengue vaccine was demonstrated inter alia in subjects from 2 to 16 years (Capeding MR, *et al.*, 2014, Lancet;384(9951):1358-65 and Villar L, *et al.*, 2015, N Engl J Med.,372(2):113-23).

A close analysis of the results obtained with this dengue vaccine however shows that it is particularly effective in the protection against dengue disease of subjects who were already seropositive at the time of the vaccination, i.e. subjects who had previously been infected by a dengue virus, irrespective of the serotype.

For subjects initially dengue naïve, i.e. who have not previously been infected by a dengue virus, the neutralizing antibody levels after the primary vaccination are however lower that the neutralizing antibody response generated in dengue immune subjects.

Based on analysis of the results from different Phase III efficacy studies, namely that higher levels of neutralizing antibodies (as measured by $PRNT_{50}$) decrease the probability of developing dengue disease, and although no absolute correlate of protection has yet been established for the dengue vaccine, it is considered that higher neutralizing antibody levels are associated with higher vaccine efficacy, i.e. a lower risk of dengue disease. In other words, $PRNT_{50}$ titres 28 days post vaccination are considered to be an inverse correlate of risk (Moodie, Z. et al., Neutralizing Antibody Correlates Analysis of Tetravalent Dengue Vaccine Efficacy Trials in Asia and Latin America, Journal of Infectious Diseases (2018), vol. 217, pages 742-753).

There is thus a need to develop a vaccine composition or a method useful in a vaccination course aiming at enhancing the neutralizing antibody response of human subjects not previously naturally infected by a dengue virus, with a view to efficiently protecting them against dengue, irrespective of the serotype.

SUMMARY OF THE INVENTION

The present invention relates to a vaccine composition for use in a method of booster vaccination for inducing in a human subject a neutralizing antibody response against dengue virus, said composition comprising a dengue antigen of at least one of serotypes 1 to 4 or a nucleic acid construct capable of expressing said antigens in the subject,

wherein said subject has previously received a primary vaccination course against each of serotypes 1 to 4 of dengue virus, and said subject was dengue naïve before said primary vaccination course, and

wherein said booster vaccination results in at least a 2-fold increase in the neutralizing antibody titre against each of serotypes 1 to 4.

The present invention further relates to a vaccine composition for use in a method of inducing in a human subject a neutralizing antibody response against dengue virus, said composition comprising a dengue antigen of each of serotypes 1 to 4, or a nucleic acid construct capable of expressing in said subject a dengue antigen of each of serotypes 1 to 4;

wherein said composition is administered as:

(a) a primary vaccination, followed by

(b) a booster vaccination,

and wherein the human subject is initially dengue naïve.

In an embodiment the invention provides a method of booster vaccination for inducing in a human subject a neutralizing antibody response against dengue virus, said method comprising administering to the subject a vaccine composition comprising a dengue antigen of each of serotypes 1 to 4 and wherein each of said dengue antigens is independently selected from the list consisting of: (a) a live attenuated dengue virus and (b) a live attenuated chimeric dengue virus,

wherein said subject has previously received a primary vaccination course against each of serotypes 1 to 4 of dengue virus, and said subject was dengue naïve before said primary vaccination course, and

wherein said vaccination composition is administered at least one year after the end of the primary vaccination course and results in at least a 2-fold increase in the neutralizing antibody titre against each of serotypes 1 to 4.

In another embodiment the invention provides a method of inducing in a human subject a neutralizing antibody response against dengue virus, comprising administering to the subject a vaccine composition comprising a dengue antigen of each of serotypes 1 to 4, wherein said dengue antigens of serotypes 1 to 4 are each independently selected from the group consisting of a live attenuated dengue virus and a live attenuated chimeric dengue virus;

wherein said vaccine composition is administered as:

(a) a primary vaccination, followed at least 1 year after the end of the primary vaccination course by

(b) a booster vaccination,

and wherein the human subject is initially dengue naive.

DEFINITIONS

The term "dengue disease", as used herein, refers to the clinical symptoms, of all grades of severity, exhibited by an individual following infection by a dengue virus. As used herein, the term dengue disease encompasses both the milder manifestations of dengue disease such as dengue fever and the more severe manifestations of dengue fever such as severe dengue as defined herein or dengue haemorrhagic fever (DHF) as defined herein. Since 1975, clinical dengue has been classified according to World Health Organization guidelines (updated in 1997) as (i) dengue fever or (ii) dengue haemorrhagic 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 1997 WHO classification, dengue fever is diagnosed by: (i) the presence of fever with at least two symptoms selected from headache, arthralgia, retro-orbital pain, rash, myalgia, haemorrhagic manifestations, and leucopenia; together with (ii) supportive serology or occurrence at the same location and time as other confirmed dengue cases. Progression to Dengue haemorrhagic fever is confirmed when fever, haemorrhagic manifestations, thrombocytopenia and evidence of plasma leakage are all observed. According to the 2009 WHO classification, diagnosis of dengue requires the presence of: (i) 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 >2 cm or an increase in haematocrit concurrent with a rapid decrease in platelet count; together with (ii) supportive serology or occurrence at the same location and time as other confirmed dengue cases. According to the 2009 WHO classification, severe dengue is defined as a diagnosis of dengue with the observation of any of the following additional events: (i) severe plasma leakage leading to shock or respiratory distress (fluid accumulation); (ii) severe bleeding as evaluated by clinicians; or (iii) severe

organ involvement (i.e. liver: AST, ALT ≥ 1000; CNS: impaired consciousness or heart or other organs).

The terms "Dengue haemorrhagic fever" or "DHF", as used herein, are consistent with the 1997 WHO definition and refer to the following symptoms – 1) Clinical manifestations: (a) Fever: acute onset, high (≥ 38°C) and continuous lasting 2 to 7 days; (b) Any of the following haemorrhagic manifestations: a positive tourniquet test, petechiae, purpura, ecchymosis, epitaxis, gum bleeding, and hematesis and/or melena; 2) Laboratory findings: (a) Thrombocytopenia (platelet count ≤ 100 x 10^9/L); (b) Plasma leakage as shown by hemoconcentration (haematocrit increased by 20% or more) or pleural effusion (seen on chest X-ray) and/or ascites and/or hypoalbuminemia. The first two clinical criteria (i.e. fever and haemorrhagic manifestations), plus thrombocytopenia and signs of plasma leakage are sufficient to establish a clinical diagnosis of DHF. Pleural effusion (seen on chest X-ray) and/or hypoalbuminemia provide supporting evidence of plasma leakage. DHF, as used herein, may be further defined on the basis of its severity. Thus 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. 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 (20 mmHg or less) or hypotension, with the presence of cold clammy skin and restlessness. Grade IV is defined as profound shock with undetectable blood pressure and 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 (i.e. temperature ≥ 38°C on at least two consecutive days) which is confirmed to be induced by a dengue virus, e.g. by reverse transcriptase polymerase chain reaction (RT-PCR) and/or by a dengue non-structural 1 (NS1) protein enzyme-linked immunosorbent assay (ELISA). In the RT-PCR method, RNA is extracted from the serum to discard potential Taq polymerase inhibitors or interfering factors, using a commercial kit. Then a dengue screen RT-PCR reaction is carried out with primers from a gene sequence conserved among dengue viruses. Results are expressed as a concentration of $\log_{10}$ plaque forming unit (PFU)/mL, by comparison with standards containing known concentrations of viral genomic nucleic acid sequences. Serotype identification of post-infectious dengue viremia is determined by testing serum samples with the Simplexa™ Dengue RT-PCR assay (Focus Diagnostics, Inc. CA, USA). Briefly, RNA is extracted from the serum to discard potential polymerase inhibitors or interfering factors, using a commercial kit. Then the Simplexa™ assay is carried out which

incorporates serotype-specific primers from dengue sequences. The results are expressed qualitatively and reported for each dengue serotype as detected or not detected. The Simplexa™ assay is used on all dengue screen RT-PCR positive or dengue NS1 Ag ELISA positive samples for serotype identification. The NS1 ELISA is performed using a commercially available kit (Platelia™ Dengue NS1 Ag, Bio-Rad, Marnes-la-Coquette, France). The manufacturer's instructions are followed. The Dengue NS1 Ag test is a one-step sandwich-ELISA based assay that enables detection of NS1 Ag in serum. The test uses murine monoclonal Abs (MAbs) for capture and revelation. Samples and controls are directly and simultaneously incubated with the conjugate within the microplate wells coated with MAb. If NS1 Ag is present in the sample, an immune-complex MAb-NS1-MAb/peroxidase will be formed. The presence of immune-complex is demonstrated by addition of a chromogenic solution that initiates a colour development reaction. After 30 minutes of incubation at room temperature, the enzymatic reaction is stopped by addition of an acid solution. The optical density (OD) reading obtained with a spectrophotometer set at 450/620 nm is proportional to the amount of NS1 Ag present in the sample. The presence of NS1 Ag in an individual sample is determined by comparing the OD reading of the sample to the OD of the cut-off control serum. Sample ratios of < 0.5, ≥ 0.5 to < 1.0, and ≥ 1 are indicative of negative, equivocal, and positive results, respectively.

The terms "severe dengue" or "severe dengue disease", as used herein refer to severe dengue as defined by the Independent Data Monitoring Committee (IDMC) established to oversee the Phase III clinical trials reported herein. According to the IDMC definition, in a case of dengue fever, the appearance of any one of the following criteria results in a diagnosis of severe dengue: (i) Shock (pulse pressure ≤ 20 mmHg in a child or adolescent, or hypotension [≤ 90 mmHg] with tachycardia, weak pulse and poor perfusion); (ii) Bleeding requiring blood transfusion; (iii) Encephalopathy i.e., unconsciousness or poor conscious state or convulsions not attributable to simple febrile convulsion or focal neurological signs. Poor conscious state or unconsciousness must be supported by Glasgow Coma Scale (GCS) score; (iv) Liver impairment (AST > 1000 U/L or prothrombin time [PT] International normalized ratio [INR] > 1.5); (v) Impaired kidney function (Serum creatinine ≥ 1.5 mg/dL) or (vi) Myocarditis, pericarditis or heart failure (clinical heart failure) supported by chest X ray (CXR), echocardiography, electrocardiogram (ECG) or cardiac enzymes where these are available. 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 severe dengue, said severe dengue need not be virologically-confirmed and may simply occur in the same location as other virologically-confirmed cases of dengue disease.

The terms "dengue fever virus", "dengue virus" 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 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. A live attenuated dengue virus may be prepared from a wild type virus, for example, by recombinant nucleic acid technology, site directed mutagenesis, serial passages on replication competent cells, chemical mutagenesis, electromagnetic radiation or genetic manipulation such as the deletion of a small section of the viral nucleic acid. Examples of live attenuated dengue viruses useful in the practice of the present invention include VDV1 (WO 2006/134433), VDV2 (WO 2006/134443), and the strains described for example in applications WO 02/066621, 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 as the dengue antigen of serotype 1 in the composition of the invention include LAV1 and VDV1. Live attenuated dengue viruses of serotype 2 which may be used as the dengue antigen of serotype 2 in the composition of the invention include LAV2 and VDV2. The term "VDV" designates 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 live attenuated dengue virus of serotype 1 known as 16007/PDK13, also called "LAV1", was derived from the wild-type DEN-1 (dengue virus serotype 1) 16007 strain by submitting the wild type strain to 13 passages through primary dog kidney (PDK) cells. LAV1 has been described in EP1159968 and has been filed with the National Microorganisms Cultures Collection (CNCM, Institut Pasteur, Paris, France) under number I-2480. "VDV1" 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 VDV1 strain has subsequently been obtained by plate purification and amplification in Vero cells. The VDV1 strain has 3 additional mutations in comparison with the DEN-1 16007/PDK13 strain. The complete nucleotide sequence of the VDV1 strain, as well as a process for preparing and characterizing the VDV1 strain have been described in international patent publication WO 2006/134433. The complete nucleic acid sequence of the VDV1 strain is as set forth in SEQ ID NO: 6.

The live attenuated dengue virus of serotype 2 known as 16681/PDK53, also called "LAV2", has been obtained from the wild-type DEN-2 (dengue virus serotype 2) 16681 strain

by submitting the wild type strain to 53 passes through PDK cells. LAV2 has been described in EP1159968 and has been filed with the National Microorganisms Cultures Collection (CNCM, Institut Pasteur, Paris, France) under number 1-2481. "VDV2" 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 VDV2 strain has subsequently been obtained by plate purification and amplification in Vero cells. The VDV2 strain has 10 additional mutations in comparison with the 16681/PDK53 strain, including 3 silent mutations and 1 mutation in a non-coding region. The complete nucleotide sequence of the VDV2 strain, as well as a process for preparing and characterizing the VDV2 strain have been described in the international patent publication WO 2006/134443. The complete nucleic acid sequence of the VDV2 strain is as set forth in SEQ ID NO: 7.

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 sequence of at least the E protein of the recipient flavivirus by the corresponding sequence of a dengue virus. Alternatively, and more preferably, the genetic backbone of the recipient flavivirus is modified by exchanging the nucleic acid sequences encoding both 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, in which case, the chimera is referred to herein as a "chimeric YF/dengue virus". Preferably, the YF backbone of a chimeric YF/dengue virus according to the present invention is from an attenuated YF virus. The recipient flavivirus may also be a dengue virus and in that case, the chimeric dengue virus is referred to herein as a "chimeric dengue/dengue virus", the dengue virus serotype characteristic of the E or the prM and E proteins being identical or different from the recipient dengue virus serotype characteristic of the genetic backbone. When the recipient flavivirus is a dengue virus, said dengue virus is preferably attenuated. When the serotypes of the recipient and donor dengue viruses are identical, the recipient dengue virus and the donor 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 chimeric YF/dengue viruses.

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). Examples of other attenuated YF 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). Advantageously, the recipient flavivirus of a live attenuated chimeric YF/dengue virus of the present invention is YF 17D or YF 17D204.

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

One example of a chimeric YF/dengue virus particularly suitable for use in the practice of the present invention is a Chimerivax® YF/dengue virus, which is also referred to herein as a "CYD" virus. As used herein, a Chimerivax® YF/dengue (or CYD) virus is a live attenuated chimeric YF/dengue virus which comprises the genomic backbone of a suitable attenuated YF virus (e.g. YF17D or YF17D204 (YF-VAX®)) 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) viruses described in WO2016/034629 have been generated by using prM and E sequences from strains DEN 1 PUO 359 (TVP1 140), DEN2 PUO 218, DEN3 PaH881/88 and DEN 4 1228 (TVP 980). For convenience, the particular Chimerivax® (CYD) viruses described in the examples of WO2016/034629 are referred to herein as "CYD1", "CYD2", "CYD3" and "CYD4". The preparation of these particular 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. The nucleotide sequences of the prM-E regions of CYD1, CYD2, CYD3 and CYD4 are set out in WO2016/034629 and in the enclosed sequence listing. 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, as described elsewhere herein, for example in the construction of other Chimerivax® YF/Dengue viruses. 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. Examples of such chimeric dengue viruses are described in WO 2011/138586.

The term "dengue virus-like particle" or "dengue VLP", as used herein, refers to a virus particle that does not contain replicative genetic material but presents at its surface a dengue E protein in a repetitive ordered array similar to the native virion structure. Typically, dengue VLPs also contain dengue prM and/or M 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, a nucleic acid construct or constructs (e.g. DNA or RNA) encoding prM/M 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 at least one viral vector. The VLP particles are then formed *in vivo*. 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 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 (Timiryasova, T.M. *et al.*, Am. J. Trop. Med. Hyg. (2013), vol. 88(5), 962-970). Briefly, neutralizing antibody titre is measured in sera collected from subjects to be tested for their level of dengue neutralising antibodies. If the subject is a vaccinated subject, a sample is collected from said subject at least 28 days following administration of a vaccine composition of the present invention. Serial, two-fold dilutions of the 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 parental dengue virus strains of the CYD dengue vaccine constructs are used as the challenge strains. The mixtures are then 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 (i.e. plaques) and a reduction in virus infectivity due to the presence of neutralising antibodies in the serum samples (i.e. a reduction in the number of plaques) 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 plaque counts) is neutralized when compared to the mean viral plaque 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 has been commonly considered that seroconversion occurs when the titre 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. However, as an alternative, a higher cut-off for determining seroconversion (i.e. a positive result) may be used in the context of the $PRNT_{50}$, for example, 25 (1/dil), 50 (1/dil), 75 (1/dil) or 100 (1/dil). As a further alternative to $PRNT_{50}$, it may be preferable to use the more stringent $PRNT_{90}$ test to assess the presence of neutralizing

antibodies against dengue. Use of the $PRNT_{90}$ may be especially preferable to assess the level of neutralising antibodies in samples obtained from subjects resident in dengue endemic areas, since the $PRNT_{90}$ test is more specific than the $PRNT_{50}$ test.

In accordance with the invention, the "seroconversion rate after a booster vaccination" of dengue vaccine refers to the percentage of subjects with either a pre-booster neutralizing antibody titer below titer 10 (1/dil) and a post-booster titer above 40 (1/dil), or a pre-booster titer above 10 (1/dil) and at least a 4-fold increase in post-booster titer as determined by $PRNT_{50}$ (Plaque Reduction Neutralization Test) immediately prior and 28 days post-booster injection, for each of the four dengue virus serotypes.

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)).

As used herein, a "dengue naïve", "dengue non-immune" or "dengue seronegative" 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 would produce a negative result in a dengue ELISA or $PRNT_{50}$ assay. An example of a dengue ELISA would be the Panbio® Dengue IgG Indirect ELISA available from Alere/Abbott. Assessment of the dengue serostatus of a subject is preferably assessed using a $PRNT_{50}$ assay. In respect of the $PRNT_{50}$ assay, a serum sample from a "dengue naïve", "dengue non-immune" or "dengue seronegative" subject would produce a result below the LLOQ of the assay.

As used herein, a "dengue immune" or "dengue seropositive" 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 would produce a positive result in a dengue ELISA or $PRNT_{50}$ assay. An example of a dengue ELISA would be the Panbio® Dengue IgG Indirect ELISA available from Alere/Abbott. Assessment of the dengue serostatus of a subject is preferably assessed using a $PRNT_{50}$ assay. In respect of the $PRNT_{50}$ assay, a serum sample from a "dengue immune" or "dengue seropositive" subject would produce a result above the LLOQ of the 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. A method of protecting, according to the present invention, 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, dengue disease caused by a dengue virus of serotype 1, dengue disease caused by a dengue virus of serotype 2, dengue disease caused by a dengue virus of serotype 3 and/or dengue disease caused by a dengue virus of serotype 4;

(ii) prevention of dengue disease, regardless of severity, caused by serotypes 1, 2, 3 and 4;

(iii) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, severe dengue disease caused by a dengue virus of serotype 1, severe dengue disease caused by a dengue virus of serotype 2, severe dengue disease caused by a dengue virus of serotype 3 and/or severe dengue disease caused by a dengue virus of serotype 4;

(iv) a reduction in the incidence or likelihood of, e.g. the prevention of, DHF caused by a dengue virus of serotype 1, DHF caused by a dengue virus of serotype 2, DHF caused by a dengue virus of serotype 3 and/or DHF caused by a dengue virus of serotype 4; preferably said reduction is statistically significant;

(v) a statistically significant reduction in the incidence or likelihood of, e.g. the prevention of, hospitalization due to: dengue disease caused by a dengue virus of serotype 1; dengue disease caused by a dengue virus of serotype 2; dengue disease caused by a dengue virus of serotype 3 and/or dengue disease caused by a dengue virus of serotype 4;

(vi) a statistically significant reduction in the incidence or likelihood, e.g. the prevention of, repeated symptomatic virologically-confirmed dengue cases due to any serotype, defined as ≥2 episodes of dengue due to different serotypes occurring more than 14 days apart.

(vii) any one of (i) to (vi) in human subjects who are at least 5 years of age;

(viii) any one of (i) to (vi) in human subjects who are at least 7 years of age;

(ix) any one of (i) to (vi) in human subjects who are at least 9 years of age;

(x) any one of (i) to (vi) in human subjects who are at least 11 years of age;

(xi) any one of (i) to (vi) in human subjects who are at least 12 years of age;

(xii) any one of (i) to (vi) in human subjects who are between 9 and 16 years of age;

(xiii) any one of (i) to (vi) in human subjects who are between 12 and 16 years of age;

(xiv) prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 60 years of age living in endemic areas;

(xv) prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 12 through 60 years of age living in endemic areas.

(xvi) prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 45 years of age living in endemic areas;

(xvii) prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 12 through 45 years of age living in endemic areas;

As used herein, a homotypic neutralizing antibody against dengue virus refers to an antibody that binds epitopes that are unique to a single serotype of dengue virus and does not

cross-react with epitopes of the 3 other serotypes. A heterotypic neutralizing antibody refers to an antibody that binds to epitopes that are conserved between at least 2 serotypes of dengue virus, such an antibody is thus a serotype cross-neutralizing antibody.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

The present inventors have demonstrated that the administration of a booster dengue vaccine unexpectedly induces an increase of the neutralizing antibody titres in subjects who were dengue naïve before the primary vaccination, which is proportionally greater than the increase in subjects who were dengue immune before said primary vaccination. Whereas the immune response to primary vaccination against dengue virus is more effective in human subjects who are dengue immune prior to the primary vaccination, the inventors have now demonstrated that the degree of modulation of the immune response to a booster vaccination is modest in this population, with respect to human subjects who have not been infected (i.e. who were dengue naïve prior to the primary vaccination) for which the degree of modulation is unexpectedly high.

According to a first aspect, the present invention thus relates to a method of booster vaccination and to a vaccine composition for use in such a method for inducing in a human subject an immune response, wherein said subject has previously received a primary vaccination against each of serotypes 1 to 4 of dengue virus and was dengue naïve before said primary vaccination, preferably was flavivirus naïve before said primary vaccination. Such a subject has preferably not previously been naturally infected by a dengue virus, and preferably not previously been naturally infected by a flavivirus.

The method of booster vaccination according to the invention comprises the step of administering the vaccine composition to the human subject.

The immune response induced by the vaccine composition of the invention or by the method of the invention is preferably a humoral response, especially a response comprising the production of neutralizing antibodies against dengue virus, i.e. a neutralizing antibody response. According to a preferred embodiment, the neutralizing antibodies are directed against each of serotypes 1 to 4 of dengue virus.

In a preferred embodiment, the method of booster vaccination and the vaccine composition for use as a booster according to this aspect of the invention induces at least a two-fold increase in the titers of neutralizing antibodies in the subjects receiving the booster, for at least one of the dengue serotypes, preferably for at least two, most preferably for at least 3, and even more preferably for each of serotypes 1 to 4, by comparison to the titers of neutralizing antibodies before the booster administration. Preferably the comparison is made between the levels measured a few days before the booster administration and around 28 days or one month after its administration. Alternatively, the level after the booster

administration is measured around one or two months after said administration, preferably between around 20 days and 60 days after said booster vaccination, especially around 28 days after said booster vaccination. The titre of neutralizing antibodies is advantageously measured by the $PRNT_{50}$ test. The neutralizing antibody titre is thus advantageously assimilated to the $PRNT_{50}$ titre in the following.

In another preferred embodiment, the vaccine composition for use as a booster and the corresponding method according to this aspect of the invention induces at least a four-fold increase in the titres of neutralizing antibodies in a human subject receiving the booster, for at least one of the dengue serotypes, preferably for at least two dengue serotypes, or more, preferably for at least serotype 4, or for serotypes 3 and 4.

The present inventors have also demonstrated that such a booster vaccination unexpectedly induces a seroconversion rate after the booster, for each serotype of the dengue virus, which is greatly increased in subjects who were dengue non-immune before the primary vaccination, with respect to the seroconversion rate in subjects who were dengue immune before the primary vaccination, by a factor of at least 2 to 5 (see table 8 of the experimental section). According to a preferred embodiment, the seroconversion rate after the booster vaccination according to the invention, in human subjects who have previously received a primary vaccination against each of serotypes 1 to 4 of dengue virus but who were dengue naïve before said primary vaccination, is at least about 30% for each serotype, preferably at least 35% for serotype 1 or at least 35% for serotype 2, at least 45% for serotype 3. The seroconversion rate after a booster vaccination is preferably estimated on a population of at least 10 different human subjects receiving the booster vaccination, preferably on a population of at least 50 subjects, even more preferably on a population of at least 100 subjects.

The inventors have demonstrated that, not only is the seroconversion rate of the booster vaccination increased in subjects who were dengue non-immune before the primary vaccination, with respect to the seroconversion rate in subjects who were dengue immune before the primary vaccination, but also that the relative rate of decline in the titre of neutralizing antibodies is lower in subjects who were dengue non-immune before the primary vaccination, with respect to the relative rate of decline in subjects who were dengue immune before the primary vaccination. Indeed, as illustrated in table 15, one year after the booster vaccination, the additive effect of the booster dose, on the titre of neutralizing antibodies, is still present in baseline naïve subjects and has disappeared in baseline immune subjects. The additive effect of the booster dose is thus more durable in baseline naïve subjects than in baseline immune subjects. More specifically, one year after the booster vaccination or more, the titre of neutralizing antibodies in the subjects receiving the booster is preferably still increased with respect to the level before the booster vaccination, preferably by a factor of at least 1.2, or at least 1.3 or more, in dengue-naïve subjects before the primary vaccination.

Such an increased titre over at least one year after the booster vaccination is for at least one serotype, preferably at least two or three, preferably for the 4 serotypes.

The vaccine composition for use in a booster vaccination according to the invention is for use in a human subject who has previously been vaccinated against dengue disease, and was flavivirus naïve before said primary vaccination. Preferably, the subject has not been naturally infected by a flavivirus, i.e. has not been infected by a flavivirus before the booster vaccination. Preferably the subject has not been previously naturally infected by a yellow fever virus, a dengue virus, irrespective of the serotype, or a Zika virus. Even more preferably, the subject to receive the booster vaccination has not been naturally infected by a dengue virus or a Zika virus. Most preferably, the human subject has not previously been naturally infected by a dengue virus, irrespective of the serotype. Before the primary vaccination, a human subject was thus dengue naïve, preferably dengue naïve and Zika naïve and even more preferably yellow fever naïve, dengue naïve and Zika naïve.

A preferred human subject is thus a subject who has, before the administration of the booster, a $PRNT_{50}$ titre against each of serotypes 1 to 4 of at least 10, (i.e. the subject is dengue immune due to the primary vaccination), but who has a $PRNT_{50}$ titre of less than 150, preferably less than 120, or preferably less than 100, preferably less than 80, preferably less than 60, preferably less than 40 or even less than 30, indicating that the subject has not been naturally infected by a dengue virus and was dengue naïve prior to the primary vaccination.

The absence of a prior natural infection by a dengue virus can also been confirmed by the absence of detection of antibodies against dengue virus antigens which may not be present in a dengue vaccine, for example antibodies against dengue non-structural protein 1 (NS1) antigen, which is absent from at least Dengvaxia®. Various tests for detecting antibodies against dengue NS1 protein are well known in the art.

A subject likely to be treated by the method of the invention, i.e. a subject who has received a primary vaccination but who has not been naturally infected, or a subject who was dengue naïve before the primary vaccination, is also characterized by the type or quality of neutralizing antibodies present in the subject. Such a subject is for example characterized as exhibiting an essentially homotypic neutralizing antibody response against only one of the 4 serotypes, and a mixed homotypic and heterotypic neutralizing antibody response against the 3 other serotypes. Preferably, the subject to be treated according to this 1st aspect exhibits an essentially homotypic neutralizing antibody response against dengue virus serotype 4, and a mixed homotypic and heterotypic neutralizing antibody response against dengue virus serotypes 1-3.

The previous dengue infection according to the present invention may be virologically-confirmed dengue disease.

The vaccine composition according to the invention comprises a dengue antigen of at least one of serotypes 1 to 4 or a nucleic acid construct capable of expressing said antigen(s) in the subject. According to a preferred embodiment, the vaccine composition comprises:

(i) a dengue antigen of at least one of serotypes 1 to 4, wherein said dengue antigen(s) of at least one of serotypes 1 to 4 is (are each independently) selected from the group consisting of:
   (a) a live attenuated dengue virus; and
   (b) a live attenuated chimeric dengue virus;

or

(ii) a nucleic acid construct(s) which is (are) able to express in said human subject a dengue antigen of at least one of serotypes 1 to 4, wherein said dengue antigen(s) is (are) dengue VLPs.

According to a preferred embodiment, the vaccine composition comprises:

(i) a dengue antigen of each of serotypes 1 to 4, wherein said dengue antigens of each of serotypes 1 to 4 are each independently selected from the group consisting of:
   (a) a live attenuated dengue virus; and
   (b) a live attenuated chimeric dengue virus;

or

(iii) a nucleic acid construct or constructs which is (are) able to express in said human subject a dengue antigen of each of serotypes 1 to 4, wherein said dengue antigens are dengue VLPs.

According to another embodiment, the vaccine composition comprises a dengue antigen, or nucleic acid able to express a dengue antigen, of least 2 serotypes, preferably at least 3 serotypes, for example serotypes 1, 2 and 4.

Preferably a vaccine composition according to the present invention comprises a dengue antigen of each of serotypes 1 to 4 which are each independently selected from the group consisting of: (a) a live attenuated dengue virus and (b) a live attenuated chimeric virus.

Preferably a vaccine composition according to the present invention comprises a dengue antigen of at least one, two, three or each of serotypes 1 to 4, wherein at least one of said dengue antigens is a live attenuated chimeric virus, preferably a live attenuated chimeric dengue virus, even more preferably a live attenuated chimeric dengue/dengue virus or a live attenuated chimeric YF/dengue virus. For example, the dengue antigen of serotype 2 is a live attenuated chimeric dengue/dengue virus. For example, a vaccine composition according to the present invention may be any of the tetravalent mixtures of dengue antigens of each of serotypes 1 to 4 (referred to as TV001, TV002, TV003 and TV004) which are disclosed in

Durbin *et al.*, Journal of Infectious Diseases (2013), 207, 957-965. Preferably, a vaccine composition according to this embodiment of the invention is TV003.

Preferably a vaccine composition according to the present invention comprises a dengue antigen of at least one, two, three or each of serotypes 1 to 4, wherein said dengue antigens are each a live attenuated chimeric dengue virus. For example, a vaccine composition of the present invention may comprise a dengue antigen of each of serotypes 1 to 4, wherein said dengue antigens of serotypes 1, 3 and 4 are each a live attenuated chimeric dengue/dengue virus and said dengue antigen of serotype 2 is a live attenuated dengue virus. For example, a vaccine composition according to the present invention may be the tetravalent mixture of dengue antigens of each of serotypes 1 to 4 (referred to as DENVax) which is disclosed in Huang *et al.*, PLoS Negl Trop Dis 7(5): e2243 (2013). Alternatively, a vaccine composition of the present invention may comprise a dengue antigen of each of serotypes 1 to 4, wherein said dengue antigens of serotypes 1, 3 and 4 are each a live attenuated chimeric YF/dengue virus and said dengue antigen of serotype 2 is a live attenuated dengue virus.

Preferably a vaccine composition according to the present invention comprises a dengue antigen of each of serotypes 1 to 4, wherein each of said dengue antigens is a live attenuated chimeric dengue virus, preferably a chimeric YF/dengue virus, more preferably a chimeric YF/dengue virus which comprises an attenuated YF genomic backbone whose prM-E sequence has been substituted with the prM-E sequence of dengue virus.

Preferably, a live attenuated chimeric dengue virus of the present invention comprises one or more proteins from a dengue virus and one or more proteins from a different flavivirus. Preferably, the different flavivirus is a yellow fever virus (i.e. a chimeric YF/dengue virus). Preferably a live attenuated chimeric dengue virus according to the present invention comprises an attenuated yellow fever virus genome whose prM-E sequence has been substituted with the prM-E sequence of a dengue virus. Alternatively, a live attenuated chimeric dengue virus of the present invention comprises one or more proteins from a first dengue virus and one or more proteins from a second dengue virus (i.e. a chimeric dengue/dengue virus). Preferably said first dengue virus and said second dengue virus are of different serotypes. Where said first dengue virus and said second dengue virus are of the same serotype, said first and second dengue viruses are different strains.

A preferred example of a dengue antigen of serotype 1 for use in the present invention is a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 1. Another preferred example of a dengue antigen of serotype 1 for use in the present invention is a live attenuated dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 6. Preferably a nucleotide sequence that has less than 100% identity to SEQ ID NO: 6 does not comprise mutations at the positions within said nucleic acid sequence which correspond to positions 1323, 1541,

1543, 1545, 1567, 1608, 2363, 2695, 2782, 5063, 5962, 6048, 6806, 7330, 7947 and 9445 of SEQ ID NO: 6.

A preferred example of a dengue antigen of serotype 2 for use in the present invention is a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 2. Another preferred example of a dengue antigen of serotype 2 for use in the present invention is a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 5. Another preferred example of a dengue antigen of serotype 2 for use in the present invention is a live attenuated dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 7. Preferably a nucleotide sequence that has less than 100% identity to SEQ ID NO: 7 does not comprise mutations at the positions within said nucleic acid sequence which correspond to positions 736, 1619, 4723, 5062, 9191, 10063, 10507, 57, 524, 2055, 2579, 4018, 5547, 6599 and 8571 of SEQ ID NO: 7.

Advantageously, a dengue antigen of serotype 2 for use in the present invention (whether said dengue antigen is, for example, a live attenuated dengue virus, a chimeric dengue virus or a VLP) comprises a Thr residue at position E-226 and/or a Val residue at position E-251. More advantageously, said dengue antigen of serotype 2 comprises a Thr residue at position E-226, a Gly residue at position E-228 and a Val residue at position E-251 In this context, E-226 designates position 226 of the Envelope (E) protein etc. The identity of an amino acid residue at a particular position can easily be determined by protein alignment, for example by alignment with the protein sequence of the E protein from CYD2, which may be easily derived from SEQ ID NO: 2.

A preferred example of a dengue antigen of serotype 3 for use in the present invention is a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 3.

A preferred example of a dengue antigen of serotype 4 for use in the present invention is a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 4.

In order to form a tetravalent dosage form of a booster composition for use according to the present invention (i.e. one containing a dengue antigen of each of serotypes 1 to 4), the preferred examples of dengue antigens of serotypes 1, 2, 3 and 4 disclosed in the preceding four paragraphs may be combined in any combination possible. Alternatively, a booster composition for use according to the present invention may be administered to a subject as bivalent dosage forms, or tetravalent dosage forms, wherein the preferred examples of dengue antigens of serotypes 1, 2, 3 and 4 disclosed in the preceding four paragraphs may be combined in any pair of bivalent or tetravalent combinations that are possible. Thus, in

particularly preferred combinations of dengue antigens of serotypes 1, 2, 3 and 4, the dengue antigens of serotypes 3 and 4 are respectively a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 3 and a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 4. In such particularly preferred combinations, the dengue antigens of serotypes 1 and 2 may respectively be:

(i) a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 1 and a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 2; or

(ii) a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 1 and a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 5; or

(iii) a live attenuated chimeric YF/dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 1 and a live attenuated dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 7 (preferably a nucleotide sequence that has less than 100% identity to SEQ ID NO: 7 does not comprise mutations at the positions within said nucleic acid sequence which correspond to positions 736, 1619, 4723, 5062, 9191, 10063, 10507, 57, 524, 2055, 2579, 4018, 5547, 6599 and 8571 of SEQ ID NO: 7); or

(iv) a live attenuated dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 6 (preferably a nucleotide sequence that has less than 100% identity to SEQ ID NO: 6 does not comprise mutations at the positions within said nucleic acid sequence which correspond to positions 1323, 1541, 1543, 1545, 1567, 1608, 2363, 2695, 2782, 5063, 5962, 6048, 6806, 7330, 7947 and 9445 of SEQ ID NO: 6 and a live attenuated dengue virus which comprises a nucleotide sequence having at least 90%, at least 95%, at least 98% or 100% sequence identity to SEQ ID NO: 7 (preferably a nucleotide sequence that has less than 100% identity to SEQ ID NO: 7 does not comprise mutations at the positions within said nucleic acid sequence which correspond to positions 736, 1619, 4723, 5062, 9191, 10063, 10507, 57, 524, 2055, 2579, 4018, 5547, 6599 and 8571 of SEQ ID NO: 7).

The vaccine composition for use according to this 1st aspect of the invention, as a booster vaccination, may advantageously be identical to the vaccine composition previously administered during the primary vaccination course. Alternatively, according to a different embodiment, the vaccine composition for use as a booster vaccination, may be different from the vaccine composition previously administered during the primary vaccination course. It may *inter alia* comprise antigen of only one serotype whereas the primary vaccination comprises antigens of each of serotypes 1-4; it may also comprise different excipients, different dosages. It may also be an entirely different vaccine composition; for example the primary vaccination is based on live attenuated chimeric dengue/dengue viruses, and the booster vaccination is based on live attenuated chimeric YF/dengue viruses.

According to a second aspect, the present invention is directed to a method of inducing in a human subject a neutralizing antibody response against dengue virus, and to a vaccine composition for use in such a method, said composition comprising a dengue antigen of each of serotypes 1 to 4, or a nucleic acid construct capable of expressing in said subject a dengue antigen of each of serotypes 1 to 4;
wherein said composition is administered as:

(a) a primary vaccination, followed by

(b) a booster vaccination,

and wherein the human subject is initially dengue naïve.

The method of inducing in a human subject a neutralizing antibody response against dengue virus according to this second aspect comprises the steps of (a) administering a vaccine composition as a primary vaccination, and of (b) subsequently administering a vaccine composition (which may be the same or different as the primary vaccination composition) as a booster vaccination.

Preferably, the dengue antigens of serotypes 1 to 4 are each independently selected from the group consisting of a live attenuated dengue virus and a live attenuated chimeric dengue virus.

Alternatively, the vaccine composition for use according this second aspect of the invention comprises one or more nucleic acid constructs capable of expressing dengue VLPs of each of serotypes 1 to 4.

All the preferred types and combinations of antigens of serotypes 1 to 4 detailed with regard to the first aspect of the invention are entirely applicable to this second aspect of the invention; *inter alia*, according to a preferred embodiment, the dengue antigens of serotypes 1 to 4 are each independently live attenuated chimeric dengue viruses, especially they are each independently selected from the group consisting of live attenuated chimeric dengue/dengue and YF/dengue viruses. According to preferred embodiments, the dengue antigens of

serotypes 1 to 4 are all live attenuated chimeric dengue/dengue viruses, or they are all live attenuated chimeric YF/dengue viruses.

Preferably, said immune response comprising the production of neutralizing antibodies against dengue virus, or said neutralizing antibody response, according to the first and second aspects of the invention results in a certain level of vaccine efficacy, preferably it is protecting the human subject against dengue disease caused by a dengue virus of at least one of serotype 1, 2, 3 and 4, and most preferably against the four serotypes. For example, a vaccine composition for use in a booster vaccination according to the present invention results in a vaccine efficacy (after booster) in respect of dengue disease caused by any serotype (in a human subject as defined herein) of at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably 60% and even more preferably 70%. For example, a vaccine composition for use in a booster vaccination according to the present invention results in a vaccine efficacy (after booster) in respect of dengue disease caused by serotype 1, serotype 2, serotype 3 or serotype 4 (in a human subject as defined herein) of at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably 60% and even more preferably 70%.

Preferably, said dengue disease caused by a dengue virus is severe dengue disease. Preferably, the method of the invention results in a reduction in the incidence or likelihood of hospitalisation due to dengue disease caused by a dengue virus, irrespective of the dengue virus serotype. Preferably, said dengue disease caused by a dengue virus is DHF.

A vaccine composition according to the 1st aspect of the present invention is administered as a booster to a human subject who has already received a primary vaccination regimen against dengue virus; the vaccine composition is thus administered to a human subject who is preferably at least 2 years old. Preferably said human subject is at least 5 years old. Preferably said human subject is at least 7 years old, even more preferably said human subject is at least 9 years old.

Most preferably, especially when the primary vaccination consists in a 3-dose regimen, administered around 6 months apart from each other, the human subject to be administered the booster dose according to the 1st aspect of the invention, is at least 10 years old, or even more preferably 11 years old. Preferably said human subject is at least 11 or 12 years old.

The vaccine composition according to the 1st aspect is administered to a subject who is preferably less than 62 years old, preferably less than 55, and even more preferably less than 47 years old. A preferred subject according to this aspect of the invention is thus aged between 2 years and 62 years, preferably between 5 years and 55 years, and more preferably between 11 years and 47 years.

A vaccine composition according to the 2nd aspect of the invention, which is to be administered as a primary vaccination followed by a booster vaccination, is to be administered to a human subject who is preferably at least 9 months old. Preferably said human subject is at least 2 years old, or 4 or 5 years old. Preferably said human subject is at least 7 years old, even more preferably said human subject is at least 9 years old.

The vaccine composition according to the 2nd aspect is administered to a subject who is preferably less than 60 years old, preferably less than 55, and even more preferably less than 45 years old. A preferred subject according to this aspect of the invention is thus aged between 9 months and 60 years, preferably between 4 years and 55 years, and more preferably between 9 years and 45 years

Alternatively, according to both aspects of the invention, said human subject is aged between 2 and 60 years old. Preferably said human subject is aged between 10 and 60 years old, for example between 10 and 50 years old. According to a preferred embodiment, said human subject is aged between 11 and 50 years old. According to an even more preferred embodiment, the subject is aged between 12 and 45 years old, for example between 12 and 30 years old.

A human subject according to the present invention is preferably not pregnant, lactating or of childbearing potential, does not have self-reported or suspected congenital or acquired immunodeficiency, has not been in receipt of immunosuppressive therapy within the 6 months prior to vaccination or systemic corticosteroids therapy for more than 2 weeks within the 3 months prior to vaccination, is not HIV seropositive and does not have systemic hypersensitivity to any of the vaccine components as defined herein.

A vaccine composition of the present invention is administered as a booster vaccination, or as a primary vaccination followed by a booster vaccination, to a human subject who is yellow fever immune or yellow fever naïve, preferably yellow fever naïve. As used herein, a yellow fever immune subject refers to a subject who has been infected by a YF virus or immunized by a YF vaccine before administration of the primary vaccination or booster composition of the present invention, i.e. a serum sample taken from said subject will produce a positive result in a YF ELISA or YF $PRNT_{50}$ assay. Conversely, a yellow fever naive subject refers to a subject who has not been infected by a YF virus or immunized by a YF vaccine before administration of the vaccine or booster composition of the invention, i.e. a serum sample taken from said subject will produce a negative result in a YF ELISA or YF $PRNT_{50}$ assay. Briefly, a YF $PRNT_{50}$ assay is carried out as follows. Serial two-fold dilutions of serum to be tested (previously heat-inactivated) are mixed with a constant concentration of the YF vaccinal strain 17D (expressed as PFU/mL). The mixtures are inoculated in duplicate into wells of a plate of confluent Vero cells. After adsorption, cell monolayers are overlaid and incubated for a few days. The reported value (end point neutralization titre) represents the highest dilution of serum at which ≥ 50% of YF challenge virus (in plaque counts) is

neutralized when compared to the negative control wells, which represents the 100% virus load. The LLOQ for the YF $PRNT_{50}$ assay is 10 (1/dil).

Preferably a vaccine composition of the present invention is administered as a booster vaccination to a human subject who is yellow fever naïve and dengue immune, more specifically to a subject who has not been infected by a YF or dengue virus, has not been immunized by a YF vaccine but has been immunized by a dengue vaccine before administration of the booster composition of the present invention.

The primary vaccination course according to both aspects of the present invention may be administered in one dose or in multiple doses, for example in one, two or three doses. When the primary vaccination consists in three doses, the first dose and the third dose are preferably administered approximately twelve months apart. For instance, a primary vaccination may consist 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 primary vaccination is administered at about three-and-a-half months after the first dose, and wherein the third dose of the primary vaccination is administered at about twelve months after the first dose).

A primary vaccination according to both aspects of the present invention may consist in two doses. Preferably, the first dose and the second dose are administered approximately about three, six, eight or nine months apart. Preferably, the second dose is administered about six months after the first dose. Alternatively, two doses may be administered to a subject simultaneously or almost simultaneously (e.g. within 24 hours of each other).

A primary vaccination according to both aspects of the present invention may also consist of a single dose.

The booster vaccination according to the invention may also be administered in one or several doses, preferably in one, two or three doses. All the different variations disclosed above with respect to the primary vaccination apply mutatis mutandis to the booster vaccination. According to a preferred embodiment, the vaccine composition of the invention is administered as a single booster dose.

Preferably, according to the present invention, the vaccine composition administered as a booster vaccination is to be administered at least one year after the end of the primary vaccination course, i.e. the first dose of the booster is administered at least one year after administration of the last dose scheduled in the initial immunization regimen, more preferably

at least two years after the primary vaccination course, and even more preferably around 4 to 6 years after the primary vaccination.

According to a preferred embodiment, the booster vaccination is administered less than 20 years after the end of the primary vaccination, i.e. the first dose of the booster is administered less than 20 years after administration of the last dose scheduled in the initial immunization regimen. For example, the booster administration is administered between 1 year and 20 years after the end of the primary vaccination course, preferably between 1.5 and 15 years after the end, more preferably between 2 years and 10 years after the end of the primary vaccination course. More preferably, the booster vaccination is administered between around 4 years after the end of the primary vaccination and about 8 years, more preferably around 4 to 5 years after the end of the primary vaccination.

In the context of the present invention, the booster vaccination may also advantageously be repeated, i.e. administered more than once, for example twice, or three times. Preferably the booster vaccination is repeated around every 4 or 5 years after the first booster vaccination, or every 7 years, or every 10 years.

A human subject according to the present invention (to which a vaccine composition is administered as a booster) is preferably resident in or travelling to a dengue endemic area. More preferably, said human subject is resident in a dengue endemic area. A human subject according to the present invention may also be resident in an area that is experiencing a dengue epidemic. The term resident is given its conventional meaning herein and refers to a person who is normally domiciled in the area in question. Dengue endemic areas are well-known to a person of skill in the art and include, according to the present invention, 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 countries or parts thereof: 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. In another particular embodiment, a dengue endemic area of the present invention may consist of the following: Brazil, Colombia and Honduras. 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, Viet Nam, Cambodia, Indonesia, Malaysia, Singapore, the Philippines, Taiwan, Papua New Guinea and Australia. A dengue endemic area according to

the present invention, (which may be national or subnational), is an area where epidemiological data indicate a high burden of disease. For example, a dengue endemic area may be defined as an area wherein the dengue seroprevalence rate in the population targeted for vaccination is at least 50%, at least 60%, at least 70%, at least 80% or at least 90%. In a preferred embodiment, a subject according to the present invention is resident in an area where the dengue seroprevalence in local population aged nine years old is at least 50%, more preferably at least 70%. In this regard, it is considered that older sub-populations exhibit greater seroprevalence rates, since as age increases, the likelihood of having been infected with a dengue virus increases.

When the vaccine composition to be used in a method according to the present invention comprises dengue antigens of serotypes 1 to 4 which comprise nucleic acid sequences having at least 90%, at least 95%, at least 98% or 100% identity to SEQ ID NOs: 1, 2, 3 and 4 respectively (for example the dengue antigens CYD1, CYD2, CYD3 and CYD4), a human subject according to the present invention (to which a vaccine composition of the present invention is administered) is advantageously resident in a dengue endemic area in which the dominant circulating strains of dengue are of serotypes 1, 3 and 4. For example, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the cases of dengue disease in said dengue endemic area are caused by a dengue virus of serotypes 1, 3 or 4. A human subject according to the present invention (to which a vaccine composition of the present invention is administered) is advantageously resident in a dengue endemic area in which the dominant circulating strains of dengue are of serotypes 3 and 4. For example, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the cases of dengue disease in said dengue endemic area are infections by a dengue virus of serotype 3 or 4.

When the vaccine composition to be used in a method according to the present invention comprises dengue antigens of serotypes 1 to 4 which comprise nucleic acid sequences having at least 90%, at least 95%, at least 98% or 100% identity to SEQ ID NOs: 1, 2, 3 and 4 respectively (for example the dengue antigens CYD1, CYD2, CYD3 and CYD4), a human subject according to the present invention (to which a vaccine composition of the present invention is administered) is advantageously resident in a dengue endemic area in which the circulating dengue strain of serotype 2 has a genotype which is characterised by the presence of Thr and Gly at positions E-226 and E-228. Advantageously, the circulating dengue strain of serotype 2 has a genotype which is characterised by the presence 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 the residues at positions E-226 and E-228 must be Thr and Gly respectively. In this context, prM-16 designates position 16 of the prM protein and E-83 designates position 83 of the E protein etc. A human subject according to the present invention (to which a vaccine composition of the present invention is administered) is preferably resident in a dengue endemic area in which the circulating

serotype 2 dengue virus has a genotype as defined in this paragraph, 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 of serotype 2 in said dengue endemic area are caused by dengue virus of serotype 2 having said genotype. Dengue disease caused by a dengue virus of serotype 2, as referred to herein, is preferably dengue disease caused by a dengue virus of serotype 2 having a genotype as defined in this paragraph.

When the vaccine composition to be used in a method according to the present invention comprises dengue antigens of serotypes 1 to 4 which comprise nucleic acid sequences having at least 90%, at least 95%, at least 98% or 100% identity to SEQ ID NOs: 1, 2, 3 and 4 respectively (for example the dengue antigens CYD1, CYD2, CYD3 and CYD4) a human subject according to the present invention (to which a vaccine composition of the present invention is administered) is advantageously resident in a dengue endemic area in which the circulating dengue strain of serotype 2 does not have an Asian-1 genotype. Dengue viruses of serotype 2 can be sub-divided into several genotypes, which are referred to as: American, Asian/American, Asian-1, Asian-2, Cosmopolitan and Sylvatic (Twiddy SS *et al.* (2002) Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. Virology; 298(1): 63-72). Thus, a human subject according to the present invention (to which a vaccine composition of the present invention is administered) is advantageously resident in a dengue endemic area in which the circulating dengue strain of serotype 2 has an American, Asian/American, Asian-2, Cosmopolitan or Sylvatic genotype. More preferably, a human subject according to the present invention (to which a vaccine composition of the present invention is administered) is advantageously resident in a dengue endemic area in which the circulating dengue strain of serotype 2 has an American, Asian/American, or Cosmopolitan genotype. A human subject according to the present invention (to which a vaccine composition of the present invention is administered) is preferably resident in a dengue endemic area in which the circulating serotype 2 dengue virus has a genotype as defined in this paragraph, 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 of serotype 2 in said dengue endemic area are caused by dengue virus of serotype 2 having an American, Asian/American, Asian-2, Cosmopolitan or Sylvatic genotype, preferably an American, Asian/American or Cosmopolitan genotype. Dengue disease caused by a dengue virus of serotype 2, as referred to herein, is preferably dengue disease caused by a dengue virus of serotype 2 having an American, Asian/American, Asian-2, Cosmopolitan or Sylvatic genotype. More preferably, dengue disease caused by a dengue virus of serotype 2, as referred to herein, is preferably dengue disease caused by a dengue virus of serotype 2 having an American, Asian/American, or Cosmopolitan genotype. The genotype of a particular dengue-2 virus strain is determined by sequence alignment and phylogenetic tree analysis. Briefly, reference sequences (which are selected nucleotide sequences encoding the E proteins of a

representative strain of each genotype as described in Twiddy *et al.*) are aligned with the nucleotide sequences encoding the E proteins of the serotype-2 strains to be genotyped. Then a phylogenetic tree is calculated and a genotype is assigned to each unknown serotype-2 strain according to their respective clustering with the reference-genotype sequences. Phylogenetic trees are calculated according to the maximum likelihood method using FastTree 2 software (Price MN *et al.*, FastTree 2--approximately maximum-likelihood trees for large alignments, PLoS One. 2010; 5(3): e9490) and the Whelan and Goldman model of amino acid evolution (Whelan S, Goldman N. A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Mol. Biol. Evol. 2001; 18(5): 691-699).

When the vaccine composition to be used in a method according to the present invention comprises dengue antigens of serotypes 1 to 4 which comprise nucleic acid sequences having at least 90%, at least 95%, at least 98% or 100% identity to SEQ ID NOs: 1, 2, 3 and 4 respectively (for example the dengue antigens CYD1, CYD2, CYD3 and CYD4), a human subject according to the present invention (to which a vaccine composition of the present invention is administered) is advantageously resident in a dengue endemic area in which the circulating dengue strain of serotype 4 is of the DEN4-II genotype. In particular, a circulating dengue strain of serotype 4 preferably has residues at “signature” positions pr73, M65, E46, E120, E160, E203, E329, E429, E455, E461 and E478 which match with the equivalent residues in the prM, M and E protein sequences of CYD4 as may be easily derived from SEQ ID NO: 4. Preferably, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or all 10 of the “signature” residues must match. M65 refers to position 65 of the M protein and pr73 refers to position 73 of the prM protein etc. A human subject according to the present invention (to which a vaccine composition of the present invention is administered) is preferably resident in a dengue endemic area in which the circulating serotype 4 dengue virus has a DEN4-II genotype as defined in this paragraph, 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 of serotype 4 in said dengue endemic area are caused by dengue virus of serotype 4 having said genotype. Dengue disease caused by a dengue virus of serotype 4, as referred to herein, is preferably dengue disease caused by a dengue virus of serotype 4 having a genotype as defined in this paragraph.

When the vaccine composition to be used in a method according to the present invention comprises dengue antigens of serotypes 1 to 4 which comprise nucleic acid sequences having at least 90%, at least 95%, at least 98% or 100% identity to SEQ ID NOs: 1, 2, 3 and 4 respectively (for example the dengue antigens CYD1, CYD2, CYD3 and CYD4), a human subject according to the present invention (to which a vaccine composition of the present invention is administered) is advantageously resident in a dengue endemic area in which the circulating dengue strains of serotypes 1, 2, 3 and 4 comprise prM-E nucleotide

sequences which have at least 90%, at least 95%, at least 98% or 100% identity to SEQ ID NOs: 1, 2, 3 and 4 respectively.

Preferably, a booster vaccination according to the present invention, i.e. a composition for use in a method according to the first or the second aspect of the present invention, reduces the incidence or likelihood of dengue disease.

Preferably, a booster vaccination according to the present invention, i.e. a composition for use in a method according to the first or the second aspect of the present invention, results in the prevention of (i.e. is for use in the prevention of) dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 60 years of age, or 9 through 45 years of age, living in endemic areas. In this context, an individual is understood to be a human subject.

Preferably, a booster vaccination according to the present invention, i.e. a composition for use in a method according to the first or the second aspect of the present invention, results in the prevention of (i.e. is for use in the prevention of) dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 12 through 60 years of age, or 12 through 45 years of age, living in endemic areas. In this context, an individual is understood to be a human subject.

The exact quantity of a live attenuated dengue virus or a live attenuated chimeric dengue virus of the present invention to be administered in a primary vaccination or in a booster vaccination may vary according to the age and the weight of the subject being vaccinated, the frequency of administration as well as the other ingredients in the composition. Generally, the quantity of a live attenuated dengue virus (e.g. VDV1 or VDV2) comprised in a dose of a vaccine composition of the present invention, for primary or booster vaccination, 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, for example about 10^4 $CCID_{50}$. The quantity of a chimeric dengue virus (such as a chimeric YF/dengue virus or a Chimerivax® (CYD) virus) comprised in a vaccine composition of the present invention, for primary or booster vaccination, lies within a range of about 10^5 $CCID_{50}$ to about 10^6 $CCID_{50}$. The quantity of a live attenuated dengue virus or live attenuated chimeric dengue virus of each of serotypes 1 to 4 comprised in a tetravalent dosage form or bivalent dosage forms according to the present invention is preferably equal. Advantageously, a vaccine composition for use according to the present invention in a primary or booster vaccination comprises an effective amount of a dengue antigen as defined herein.

A vaccine composition for use in a booster vaccination according to the 1st aspect of the present invention, or for use in a primary vaccination followed by a booster vaccination according to the 2nd aspect, 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.

A vaccine composition for use as a booster 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.

As appreciated by skilled artisans, a vaccine composition for use as a booster according to the first or 2nd aspect 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 for use according to the first or 2nd aspect 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 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.

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 as a booster vaccination according to the first aspect of the invention, or as a primary vaccination and a booster vaccination according to the 2nd aspect, in a method of protecting a human

subject against dengue disease. The kit may comprise said vaccine composition in the form of a single tetravalent dosage form or said kit may comprise said vaccine composition in the form of two bivalent dosage forms. 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 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, as a booster vaccination or as a primary vaccination and a booster vaccination. The leaflet may further mention the vaccination regimen and the human subject population to be vaccinated, namely subjects who have not previously been naturally infected by a dengue virus, irrespective of the serotype.

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

(i) the number of cases of dengue disease caused by dengue virus of any serotype;

(ii) the number of severe dengue cases caused by dengue virus of any serotype;

(iii) the number of DHF cases caused by dengue virus of any serotype; and/or

(iv) the number of hospitalized cases of dengue disease caused by dengue virus of any serotype;

in a group of subjects that has received said booster composition, and comparing those measurements with the equivalent measurements from a control group of subjects that has not received said booster composition, wherein the subjects in both said groups are resident in a dengue endemic region, have received a primary vaccination and have not been naturally infected by a dengue virus. A statistically conclusive reduction in any one or more of (i) to (iv) in the group of subjects receiving the booster when compared with the control group of subjects not receiving the booster, is indicative of the efficacy of a booster composition according to the present invention.

The efficacy of a booster 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 booster composition (e.g. after administration of one, two or three doses of said booster 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 booster composition and who have developed virologically-confirmed dengue disease after the administration of the booster, and comparing those measurements with the equivalent measurements from a control group of subjects that has not received said booster composition and who have developed virologically-confirmed dengue disease, wherein the subjects in both said groups have received a primary vaccination and have not been naturally infected by a dengue virus before the booster. A statistically significant reduction in any one or more of (i) to (iv) in the group of subjects who have received the booster dose and have developed virologically-confirmed dengue disease when compared with the control group of subjects who have not received the booster and have developed virologically-confirmed dengue disease is indicative of the efficacy of a booster composition according to the present invention in reducing the severity or likelihood of dengue disease.

The efficacy of a booster 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 booster composition (e.g. after administration of one, two or three doses of said booster composition) the neutralising antibody titre induced by said booster composition in a group of subjects that has received said booster composition and using a correlate of risk or a correlate of protection (if available) to convert the neutralizing antibody titre into a measure of efficacy.

Alignments of the nucleic sequences disclosed herein with other nucleic acid sequences may be achieved by any of the suitable sequence alignment methods well known to a person skilled in the art. For example, sequence alignments may be carried out by hand. More conveniently, an alignment may be carried out using a specialised computer program. For example, optimal sequence alignment can be achieved and percent identity can be determined by global sequence alignment algorithms such as the Multiple Sequence Alignment (MSA) algorithms Clustal W and Clustal Omega algorithms, or the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm (Edgar RC, Nucl. Acids Res. (2004): 32 (5) : 1792). These algorithms are available on the European Bioinformatics Institute (EBI) web site at http://www.ebi.ac.uk/services. Where such algorithms have user-defined parameters, the default parameters should be used.

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.

EXPERIMENTAL SECTION

CYD64 clinical trial: Immunogenicity and Safety of a Tetravalent Dengue Vaccine Given as a Booster Injection in Adolescents and Adults Who Previously Completed the 3-dose Schedule in a Study Conducted in Latin America.

1) Summary

The aim of the study is to assess and describe the booster effect of a CYD dengue vaccine dose administered 4 to 5 years after the completion of a 3-dose vaccination schedule.

Primary Objective

- To demonstrate the non-inferiority, in terms of geometric mean of titer ratios (GMTRs), of a CYD dengue vaccine booster compared to the third CYD dengue vaccine injection in subjects from previous CYD dengue vaccine trials.

Secondary Objectives:

- If the primary objective of non-inferiority is achieved: To demonstrate the superiority, in terms of GMTRs, of a CYD dengue vaccine booster compared to the third CYD dengue vaccine injection in subjects from previous CYD dengue vaccine trials.
- To describe the immune responses elicited by a CYD dengue vaccine booster and placebo injection in subjects who received 3 doses of the CYD dengue vaccine in previous CYD dengue vaccine trials.
- To describe the neutralizing Antibodies (Ab) levels of each dengue serotype post-dose 3 (previous CYD dengue vaccine trials' subjects) and immediately prior to booster or placebo injection in all subjects.
- To describe the neutralizing Ab persistence 6 months and 1 year post booster or placebo injection in all subjects.
- To evaluate the safety of booster vaccination with the CYD dengue vaccine in all subjects.

Primary Outcome Measures:

- Neutralizing antibody levels against each dengue virus serotype measured 28 days after the third CYD dengue vaccine injection and 28 days after the booster injection in the study group [Time Frame: Day 28 post booster vaccination].

Secondary Outcome Measures:

- Neutralizing antibody levels against each of the 4 parental dengue virus strains of the CYD dengue vaccine immediately prior and 28 days post booster or placebo injection [Time Frame: Before and Day 28 post booster vaccination]
- Neutralizing antibody levels against each of the 4 parental dengue virus strains of the CYD dengue vaccine 6 months and 1 year post booster or placebo injection [Time Frame: 6 months and 12 months post booster vaccination]
- Percentages of subjects with seroconversion 28 days after the booster injection for each of the four parental dengue virus strain of CYD dengue vaccine: percentages of subjects with either a pre-booster titer < 10 (1/dil) and a post-booster titer ≥ 40 (1/dil), or a pre-booster titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-booster titer as determined by PRNT immediately prior and 28 days post-booster or placebo injection [Time Frame: Before and Day 28 post booster vaccination]
- Number of participants reporting solicited injection site reactions, solicited systemic reactions, unsolicited adverse events, and serious adverse events occurring during trial [Time Frame: Day 0 up to 2 years post vaccination]
  Solicited injection site reactions: Pain, Erythema, and Swelling. Solicited systemic reactions: Fever (temperature), Headache, Malaise, Myalgia, and Asthenia

Neutralizing antibody levels against each dengue virus serotype are measured using dengue plaque reduction neutralization test (PRNT).

2) Statistical methods

Hypothesis and Statistical Method for the Primary Objective (Group 1 only)

Hypotheses:

Individual Hypotheses for Each Serotype:

A non-inferiority testing approach was performed for each serotype to demonstrate the non-inferiority, in terms of GMTRs, 28 days post-injection, of a CYD dengue vaccine booster dose compared to the third CYD dengue vaccine dose in subjects from CYD13 (Villar LA, *et al*, 2013, Pediatr Infect Dis J;32(10):1102-1109) and CYD30 (Dayan GH, *et al*, 2013, Am J Trop Med Hyg 2013; 89(6): 1058–1065) trials.

Individual hypotheses for each serotype were as follows:

$$H_0^i : GM(V_{Booster}^i / V_{PD3}^i) \leq 1/2$$

$$H_1^i : GM(V_{Booster}^i / V_{PD3}^i) > 1/2$$

Where i = 1, 2, 3 and 4 ; $V_{Booster}^i$ is the immunogenicity titer 28 days after the CYD dengue vaccine booster dose and V_{PD3}^i is the immunogenicity titer 28 days after the third CYD dengue vaccine dose in CYD13 and CYD30 subjects.

Overall Hypothesis:

The overall null hypothesis can be stated as for at least 1 serotype, the post booster dose response (28 days after the CYD dengue vaccine booster injection) is inferior to the PD3 response (28 days after the third CYD dengue vaccine dose in CYD13 and CYD30subjects)

H_0^G at least one H_0^i not rejected

H_1^G : all H_0^i are rejected

Statistical Methods

A non-inferiority test was performed using the 95% two-sided CI of GM($V_{Booster}$ / V_{PD3}) for each serotype; the 95% CI was calculated using paired t-test.

Subjects with non-missing PD3 and post booster dose titer were included in this analysis.

For each serotype, non-inferiority was demonstrated if the lower limit of the two-sided 95% CI was greater than 1/2. If the null hypothesis is rejected, then the alternative hypothesis of non-inferiority is supported.

The overall null hypothesis was rejected if the four individual null hypotheses were rejected simultaneously.

Hypotheses and Statistical Methods for the First Secondary Objective

As non-inferiority was demonstrated for the primary endpoint, then superiority hypotheses will be performed.

Hypotheses:

Individual Hypotheses for Each Serotype:

A superiority hypothesis testing approach was performed for each serotype to demonstratethe superiority, in terms of GMTRs, 28 days post-injection, of a CYD dengue vaccine booster dose compared to the third CYD dengue vaccine dose in subjects from CYD13 and CYD30 trials. Individual hypotheses for each serotype will be as follows:

$H_0^i : GM(V_{Booster}^i / V_{PD3}^i) \leq 1$

$H_1^i : GM(V_{Booster}^i / V_{PD3}^i) > 1$

Where i = 1, 2, 3 and 4 ; $V_{Booster}^i$ is the immunogenicity titer 28 days after the CYD dengue vaccine booster dose and V_{PD3}^i is the immunogenicity titer 28 days after the third CYD dengue vaccine dose in CYD13 and CYD30 subjects.

Overall Hypothesis:

The overall null hypothesis can be stated as for at least 1 serotype, the post booster dose response (28 days after the CYD dengue vaccine booster injection) is not superior to the PD3 response (28 days after the third CYD dengue vaccine dose in CYD13 and CYD30 subjects).

H_0^G at least one H_0^i not rejected

H_1^G : all H_0^i are rejected

Statistical Methods

A superiority test was performed using the 95% two-sided CI of GM($V_{Booster}$ / V_{PD3}) for each serotype; the 95% CI was calculated using paired t-test.

Subjects with non-missing PD3 and post booster dose titer were included in this analysis.

For each serotype, superiority was to be demonstrated if the lower limit of the two-sided 95% CI is greater than 1. If the null hypothesis was rejected, then the alternative hypothesis of superiority was supported.

The overall null hypothesis was to be rejected if the four individual null hypotheses were rejected simultaneously.

Statistical Methods for Other Secondary and Additional Objectives

All other analyses are descriptive; no hypotheses are tested.

For immunogenicity, 2 sample t-test on the log_{10} transformed titers were used for 95% CI for the ratio of geometric mean titers (GMTs) (difference between GMTs on log scale).

The 95% CIs for percentages were calculated using the exact binomial distribution (Clopper-Pearson's method). Assuming that log_{10} transformation of the titers/titers ratio followed a normal distribution, first, the mean and 95% CIs were calculated on log_{10} (titers/ titers ratio) using the usual calculation for normal distribution, then antilog transformations were applied to the results of calculations, to compute GMTs/GMTRs and their 95% CIs.

For safety, the exact binomial distribution (Clopper-Pearson method) for proportions was used in calculations of the 95% CIs.

Calculation of sample size:

There was to be 279 subjects in Group 1 and 93 subjects in Group 2. Assuming a dropout rate of approximately 2% for each group 28 days post injection, a total of 273 and 91 evaluable subjects was anticipated for Groups 1 and 2, respectively. With 273 evaluable subjects, the probability of observing at least 1 AE with true incidence of 1.1% was approximately 95%.

Sample size for the primary endpoint (only for Group 1) was estimated to demonstrate non-inferiority, in terms of GMTRs, 28 days post-injection, of a CYD dengue vaccine booster compared to the third CYD dengue vaccine dose in subjects from CYD13 and CYD30 trials.

With 273 evaluable subjects in Group 1, the overall power (see Table 1) using paired t-test to reject the 4 individual null hypotheses simultaneously was expected to be 88.3%; calculation assumed a non-inferiority margin (delta) =2, one-sided type I error =0.025 and correlation between the responses PD3 and post booster dose of the same serotype in the same subject =0.5.

Table 1: Power/Sample size calculation summary table for primary endpoint

Component (Antigen)	Standard deviation (log 10)	Non-Inferiority Definition	Power for N=273
Serotype 1	(sd1=0.88,sd2=1.76)	> 1/2	0.902
Serotype 2	(sd1=0.70,sd2=1.40)	> 1/2	0.983
Serotype 3	(sd1=0.62,sd2=1.24)	> 1/2	0.996
Serotype 4	(sd1=0.50,sd2=1.00)	> 1/2	1.000
Overall			**0.883**

The calculation of the standard deviation for PD3 (sd1) was based on the weighted average of 28-day PD3 standard deviations of titers from the Phase II trials CYD13 and CYD30 and the standard deviation for post booster dose (sd2) was estimated based on standard deviation for PD3.

Since 4 individual null hypotheses should be rejected simultaneously to reject the overall null hypothesis, no multiplicity adjustment for alpha is necessary.

A 3:1 randomization ratio between Group 1 and Group 2 was chosen, so 279 and 93 subjects were expected to be enrolled in Group 1 and Group 2, respectively.

Calculation of Geometric Mean of Titer (GMT) and Geometric Mean of Titer Ratios (GMTR):

The geometric mean of the neutralizing antibody titer was calculated assuming that Log10 transformation of the titers follows a normal distribution, such that the mean and the 95% CI were calculated on Log10 (titers) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom). The antilog transformation is then applied to the results of calculations, in order to provide geometric mean of titers (GMTs).

For the computation of GMTs, a titer reported as below LLOQ is converted to a value of 0.5 LLOQ.

The GM is defined as follows:

$$GM = \left(\prod_{i=1}^{n} y_i\right)^{1/n} = 10^{\wedge}\left(\frac{1}{n}\sum_{i=1}^{n}\log_{10}(y_i)\right)$$

With respect to Geometric Mean Ratios, they were obtained by first calculating the difference of the log transformed data between two comparable groups, and then the ratios are obtained by anti-log transformation of the difference.

For calculating the geometric mean titer ratio (GMTR), the values below LLOQ are converted to 0.5 LLOQ for a numerator, and the values below LLOQ are converted to LLOQ for a denominator.

This method provides the most conservative results for GMTR.

3) **Method for assessing the dengue neutralizing antibody level and seroconversion:**

Dengue neutralizing Ab levels are measured by PRNT (using parental dengue virus strains of CYD dengue vaccine constructs) by Sanofi Pasteur GCI, Swiftwater, USA (or outsourced with a GCI selected external laboratory).

Serial, 2-fold dilutions of serum to be tested (previously heat-inactivated) are mixed with a constant challenge-dose of each dengue virus serotype 1, 2, 3 or 4 (expressed as plaque-forming unit [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 formation of plaques. A reduction in virus infectivity due to neutralization by Ab present in serum samples is detected. The reported value (end point neutralization titer) represents the highest dilution of serum at which ≥ 50% of dengue challenge virus (in plaque counts) is neutralized when compared to the mean viral plaque count in the negative control wells which represents the 100% virus load. The end point neutralization titers are presented as discontinuous values. The lower limit of quantitation (LLOQ) of the assay is 10 (1/ dil).

Seroconversion rates 28 days after the booster injection for each of the four parental dengue virus strain of CYD-TDV dengue vaccine, was defined as the percentages of subjects with either a pre-booster titer < 10 (1/dil) and a post-booster titer ≥ 40 (1/dil), or a pre-booster titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-booster titer as determined by PRNT50 (Plaque Reduction Neutralization Test) immediately prior and 28 days post-booster or placebo injection. The safety profile of the booster dose was also analyzed with no specific findings.

4) **Introduction:**

CYD64 is a multi-center, observer-blind, randomized, placebo controlled, Phase II non-inferiority trial conducted in 251 healthy adolescents and adults in Brazil, Colombia, Honduras, Mexico and Puerto Rico, who received one CYD-TDV dengue vaccine (Dengvaxia®) booster dose between April 14, 2016 and October 19, 2016. It was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation guidelines for good clinical practice as well as with all local and/or national regulations. In addition, each study site's Institutional Review Board and Independent Ethics Committee approved the study protocol. No protocol amendments have been done to date. Written informed consent was obtained from all participants and/or participants' parents/guardians before study entry.

Eligible participants were healthy adolescents and adults aged 15.3 – 23.8 years that had received 3 doses of the CYD-TDV dengue vaccine 4-5 years earlier in two previous specific trials (CYD30 and CYD13, NCT01187433 and NCT00993447 respectively). Exclusion criteria included previous vaccination against dengue that was not part of the previous mentioned trials; pregnant, lactating or childbearing potential women; participation at any time of study

enrollment in another trial; reception of any vaccine in the 4 weeks preceding the trial vaccination or planned to receipt any vaccine in the 4 week following the trial vaccination; reception of immune globulins, blood or blood- derived products in the past 3 months; known or suspected congenital or acquired immunodeficiency; reception of any immunosuppressive therapy; known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccines used in the trial or to a vaccine containing any of the same substances; chronic illness that, in the opinion of the Investigator, could interfere with trial conduct or completion; deprivation of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily; current alcohol abuse or drug addiction; moderate or severe acute illness/infection on the day of vaccination or febrile illness (temperature ≥ 38.0°C); identified as an Investigator or employee of the Investigator or study center with direct involvement in the proposed study, or any identified immediate family member.

Each of the 251 participants enrolled in the trial were randomly assigned to one of the two study groups (group 1 or group 2) via an interactive voice response system or interactive web response system according to a 3:1 ratio (3 subjects in the CYD-TDV Dengue Vaccine Group for 1 subject included in the Placebo Group). Randomization was performed with permuted block method with stratification by site. A double randomization system was used, this implies that the subject treatment allocation was separated from doses dispensing. The unique dose numbers was defined according to a random list to ensure that dose numbers could not be used to distinguish between treatment groups. Subject numbers were not reassigned for any reason.

All participants in Group 1 received a CYD-TDV Dengue vaccine and all participants in Group 2 received a placebo injection at enrollment (day 0); also all participants provided 1 pre-injection blood sample at enrollment to assess baseline dengue immune status before the first vaccination, and 1 blood sample 28 days post-injection for dengue immunogenicity. Neutralizing antibodies against each of the 4 parental dengue virus strains were measured 28 days after the third CYD-TDV dengue vaccine injection and 28 days post-booster injection (Group 1 only).

For both groups, neutralizing antibodies (Nabs) against each of the 4 parental dengue virus strains were measured immediately prior the booster or placebo injection. Also, individual post-booster/pre-booster Geometric Mean Titers Ratios (GMTRs) for each of the four parental dengue virus strains of the CYD-TDV dengue vaccine were measured immediately prior and 28 days post-booster or placebo injection.

For both groups, Nabs against each of the 4 parental dengue virus strains were measured 6 months and one year post booster or placebo injection.

5) Results of the CYD64 trial, 28 days post-booster dose

A total of 251 were randomized out of the 372 planned subjects. Following randomization, 187 subjects were allocated to the CYD-TDV Dengue Vaccine Group and 64 subjects to the Placebo Group. The overall distribution of randomized subjects by country and treatment group is summarized in table 2.

Table 2. Subjects randomized per country

	CYD Dengue Vaccine Group (N=187)	Placebo Group (N=64)	All (N=251)
Country	**n (%)**	**n (%)**	**n (%)**
All	187 (100.0)	64 (100.0)	251 (100.0)
Brazil	32 (17.1)	11 (17.2)	43 (17.1)
Colombia	57 (30.5)	19 (29.7)	76 (30.3)
Honduras	32 (17.1)	10 (15.6)	42 (16.7)
Mexico	49 (26.2)	18 (28.1)	67 (26.7)
Puerto Rico	17 (9.1)	6 (9.4)	23 (9.2)

Overall, at 28 days post-booster injection, 250 (99.6%) subjects were present (i.e.1 subject from the CYD-TDV Dengue Vaccine Group was absent) and 249 (99.2%) subjects provided a blood sample (i.e., 2 subjects from the CYD-TDV Dengue Vaccine Group did not provide a sample). At 28 days post-booster injection there was only 1 (0.5%) subject who was discontinued from the study. The reason for discontinuation was non-compliance with the protocol.

Non-inferiority of CYD-TDV dengue vaccine booster compared to the 3rd CYD-TDV dengue vaccine dose in previous trials.

Non-inferiority of dengue Nab after CYD-TDV dengue vaccine booster dose compared to the 3rd CYD-TDV dengue vaccine dose (PD3) in terms of Dengue PRNT was demonstrated for the 4 serotypes (lower limit of 2-sided 95% CI greater than 1/2). A Post booster/PD3 ratio with a 95%CI was calculated for each serotype. (Table 3) A covariance analysis of post booster titers against each of the 4 serotypes was done for controlling the baseline Nab levels (removing pre booster effect); a Dengue/Placebo GMT ratio with a 95%CI was calculated for each serotype: serotype 1, ratio 2.04 (1.50;2.78), p value 0.0004; serotype 2, ratio 1.74 (1.28;2.38), p value 0.0021; serotype 3, ratio 1.85 (1.37;2.50), p value 0.0002; and serotype 4, ratio 2.19 (1.53;3.13), p value <0.0001. This shows that the immunological response is better in the vaccinated group compared with placebo. (Table 4).

Superiority of CYD-TDV dengue vaccine booster compared to the 3rd CYD-TDV dengue vaccine dose in previous trials.

As the overall non-inferiority of the CYD-TDV dengue vaccine booster could be demonstrated, a superiority analysis of the booster dose compared to the third dose of the selected previous trials was performed for each serotype using GMTRs. The superiority of the booster dose was demonstrated for serotype 1, serotype 2, and serotype 4. The superiority of the booster dose could not be demonstrated for serotype 3 as the lower limit of the two-sided 95% CI of the GMTR was < 1 for this serotype. (Table 5).

Immune response 28 days post-booster injection

At pre-booster injection, GMTs were comparable between treatment groups. They also tended to be within a similar range for serotype 1, serotype 2 and serotype 3. The GMTs of serotype 4 were lower in both groups. After the CYD-TDV dengue vaccine booster injection, GMTs increased as compared to pre-booster injection level. After the placebo injection, pre-booster injection seropositivity rates per serotype tended to remain stable after placebo injection. (Table 6).

Table 3. Non-inferiority of CYD-TDV dengue vaccine booster dose compared to the third CYD-TDV dengue vaccine dose from CYD13 or CYD30 - Dengue PRNT - Per-Protocol Analysis Set

	Post dose 3 in CYD13 and CYD30 (PD3) (N=177)			Post booster dose in CYD64 (V04) (N=177)			Ratio (Post booster/PD3)			Non-inferiority
Component	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)	
Serotype 1 [PRNT – 1/dil]	176	316	(233; 428)	177	560	(421; 744)	176	1.66	(1.33; 2.06)	Yes
Serotype 2 [PRNT – 1/dil]	175	356	(275; 462)	177	657	(520; 830)	175	1.82	(1.43; 2.31)	Yes
Serotype 3 [PRNT – 1/dil]	175	640	(516; 794)	177	671	(535; 843)	175	1.04	(0.841; 1.27)	Yes
Serotype 4 [PRNT – 1/dil]	176	243	(195; 303)	177	344	(279; 424)	176	1.32	(1.01; 1.74)	Yes

M: number of subjects with available data at both time points
For each serotype, non-inferiority was demonstrated if the lower limit of the two-sided 95% CI for the ratio is greater than ½. Overall non-inferiority will be demonstrated if all 4 serotypes achieve non-inferiority

Table 4. Analysis of covariance of post-booster titers against each of the four serotypes with the parental dengue virus strains - Dengue PRNT - Per-Protocol Analysis Set.

	CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)			Difference (Dengue – Placebo)		Ratio (Dengue/Placebo)		p value for baseline*group interaction term*	p value for group*country interaction term*
Component	**M**	**LSMEAN**	**(95% CI)**	**M**	**LSMEAN**	**(95% CI)**	**LSMEAN**	**(95% CI)**	**GM**	**(95% CI)**		
Serotype 1 [PRNT – 1/dil]	177	2.74	(2.67; 2.82)	64	2.43	(2.32; 2.55)	0.310	(0.176; 0.445)	2.04	(1.50; 2.78)	0.0004	0.3250
Serotype 2 [PRNT – 1/dil]	177	2.79	(2.71; 2.86)	64	2.55	(2.43; 2.66)	0.242	(0.107; 0.376)	1.74	(1.28; 2.38)	0.0021	0.7881
Serotype 3 [PRNT – 1/dil]	177	2.86	(2.79; 2.93)	64	2.59	(2.48; 2.71)	0.266	(0.135; 0.398)	1.85	(1.37; 2.50)	0.0002	0.1830
Serotype 4 [PRNT – 1/dil]	177	2.55	(2.47; 2.64)	64	2.21	(2.08; 2.35)	0.341	(0.186; 0.495)	2.19	(1.53; 3.13)	0.0001	0.6498

M: number of subjects available for the endpoint
LSMEAN: least squares of mean
Difference in LSMEANS and 95% CI were calculated using the analysis of covariance with pre-booster titer value and country as covariates without any interaction term
* p value for the interaction terms were derived from the analysis of covariance on post-booster titers with pre-booster titer value and country as covariates with the interaction term between the pre-booster titers and the randomized group, plus the interaction term between country and the randomized group.

Table 5. Superiority of CYD-TDV dengue vaccine booster dose compared to the third CYD-TDV dengue vaccine dose from previous trials - Dengue PRNT - Full Analysis Set

	Post dose 3 in CYD13 and CYD30 (PD3) (N=185)			Post booster dose in CYD64 (V04) (N=185)			Ratio (Post booster/PD3)			
Component	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)	Superiority
Serotype 1 [PRNT – 1/dil]	184	302	(224; 406)	185	536	(404; 710)	184	1.66	(1.34; 2.06)	Yes
Serotype 2 [PRNT – 1/dil]	183	340	(264; 439)	185	653	(519; 823)	183	1.90	(1.49; 2.41)	Yes
Serotype 3 [PRNT – 1/dil]	183	611	(495; 755)	185	662	(529; 827)	183	1.07	(0.870; 1.31)	No
Serotype 4 [PRNT – 1/dil]	184	239	(193; 295)	185	347	(283; 426)	184	1.37	(1.05; 1.78)	Yes

M: number of subjects with available data at both time points

For each serotype, superiority will be demonstrated if the lower limit of the two-sided 95% CI for the ratio is greater than 1. Overall superiority will be demonstrated if all 4 serotypes achieve superiority

Table 6. Summary of geometric means of titers and geometric means of individual titer ratios of antibody against each serotype with the parental dengue virus strains at pre- and post-booster injection - Dengue PRNT - Per-Protocol Analysis Set

		CYD Dengue Vaccine Group (N=177)			**Placebo Group (N=64)**		
Component	**Time point/ratio**	**M**	**GM**	**(95% CI)**	**M**	**GM**	**(95% CI)**
Serotype 1 [PRNT - 1/dil]	V01 (D0)	177	325	(233; 452)	64	349	(201; 607)
	V04 (D28)	177	560	(421; 744)	64	297	(162; 547)
	Ratio V04 (D28) /V01 (D0)	177	1.59	(1.33; 1.90)	64	0.798	(0.623; 1.02)
Serotype 2 [PRNT - 1/dil]	V01 (D0)	177	360	(267; 484)	64	323	(195; 535)
	V04 (D28)	177	657	(520; 830)	64	354	(205; 610)
	Ratio V04 (D28) /V01 (D0)	177	1.70	(1.43; 2.03)	64	1.03	(0.754; 1.40)
Serotype 3 [PRNT - 1/dil]	V01 (D0)	177	357	(269; 472)	64	442	(270; 724)
	V04 (D28)	177	671	(535; 843)	64	432	(266; 700)
	Ratio V04 (D28) /V01 (D0)	177	1.78	(1.47; 2.16)	64	0.946	(0.749; 1.19)
Serotype 4 [PRNT - 1/dil]	V01 (D0)	177	162	(134; 195)	64	161	(108; 242)
	V04 (D28)	177	344	(279; 424)	64	161	(110; 237)
	Ratio V04 (D28) /V01 (D0)	177	2.09	(1.65; 2.63)	64	0.946	(0.777; 1.15)

M: number of subjects available for the endpoint

The seroconversion rates for 3 of the 4 serotypes were meaningfully different between treatment groups 28 days post-booster injection. The seroconversion rate for serotype 1 was 16.9% (95% CI: 11.7; 23.3) in CYD-TDV Dengue Vaccine Group and 3.1% (95% CI: 0.4; 10.8) in the Placebo Group; for serotype 2, it was 19.2% (95% CI: 13.7; 25.8) and 17.2% (95% CI: 8.9; 28.7); for serotype 3, the seroconversion rate was 20.3% (95% CI: 14.7; 27.0) and 4.7% (95% CI: 1.0; 13.1); and for serotype 4, it was 19.8% (95% CI: 14.2; 26.4) and 6.3% (95% CI: 1.7; 15.2), respectively. One of the plausible explanations of the high seroconversion rate against serotype 2 in the Placebo Group as compared to the CYD-TDV Dengue Vaccine Group is the impact of a natural infection booster effect on GMTs.

Dengue serostatus at baseline

The immune response to CYD-TDV dengue vaccine booster injection was analyzed according to the serostatus of subjects at baseline (i.e. at D0 in the previous trials CYD13 and CYD30). Among the 177 subjects enrolled in the CYD-TDV Dengue Vaccine Group, 136 (77%) subjects were dengue-immune at baseline and 41 (23%) were dengue non-immune. In the Placebo Group, there were 46 subjects dengue-immune at baseline and 18 non-immune subjects. Overall, NAb titers against each serotype at PD3, at pre-booster injection, as well as 28 days post-booster injection, were higher in subjects dengue-immune at baseline (Tables 7A and 7B).

Dengue serostatus at pre-booster injection

At pre-booster injection, GMTs (1/dil) ranged from 224 (serotype 4) to 668 (serotype 1) in dengue-immune subjects and from 29.6 (serotype 1) to 54.9 (serotype 4) in dengue non-immune subjects. At 28 days post-booster injection, GMTs (1/dil) ranged from 343 (serotype 4) to 940 (serotype 1) in dengue-immune subjects and from 100 (serotype 1) to 347 (serotype 4) in dengue non-immune subjects. Dengue serostatus at baseline had a meaningful difference in the seroconversion rate against each serotype. The seroconversion rates were higher in the dengue non-immune group of subjects. (Table 8).

Safety Evaluations

After the CYD-TDV dengue vaccine or the placebo injection, all subjects were assessed for immediate reactions, solicited reactions and unsolicited events or reactions. SAEs were collected throughout the study and serious and non-serious AESIs were collected in defined time-windows according to the type of AESI. An overview of the safety and reactogenicity up to 28 days post-booster injection is provided in table 9.

TABLE 7A – NAb titres against each serotype at PD3, pre-booster injection and 28 days post-booster injection in subjects dengue naïve (non-immune) at baseline (i.e. at D0 in the previous trials CYD13 and CYD30)

		CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)		
Component	**Time point/ratio**	**M**	**GM**	**(95% CI)**	**M**	**GM**	**(95% CI)**
Serotype 1 [PRNT - 1/dil]	Post dose 3 in CYD13 and CYD30 (PD3)	40	26.2	(17.0; 40.4)	18	39.9	(19.3; 82.4)
	V01 (D0)	41	29.6	(15.6; 56.1)	18	54.0	(21.4; 136)
	V04 (D28)	41	100	(52.5; 192)	18	40.4	(12.6; 130)
	Ratio V01 (D0) /PD3	40	0.801	(0.431; 1.49)	18	1.16	(0.524; 2.57)
	Ratio V04 (D28) /PD3	40	2.86	(1.48; 5.51)	18	0.866	(0.318; 2.36)
	Ratio V04 (D28) /V01 (D0)	41	2.54	(1.57; 4.11)	18	0.616	(0.356; 1.07)
Serotype 2 [PRNT - 1/dil]	Post dose 3 in CYD13 and CYD30 (PD3)	40	57.1	(39.6; 82.3)	18	79.9	(39.3; 163)
	V01 (D0)	41	48.9	(25.4; 94.1)	18	55.0	(20.2; 150)
	V04 (D28)	41	213	(121; 375)	18	61.0	(17.5; 213)
	Ratio V01 (D0) /PD3	40	0.757	(0.416; 1.38)	18	0.637	(0.201; 2.03)
	Ratio V04 (D28) /PD3	40	3.45	(1.93; 6.18)	18	0.706	(0.181; 2.75)
	Ratio V04 (D28) /V01 (D0)	41	3.38	(2.23; 5.12)	18	0.880	(0.415; 1.86)
Serotype 3 [PRNT - 1/dil]	Post dose 3 in CYD13 and CYD30 (PD3)	41	129	(98.1; 168)	18	138	(82.0; 234)
	V01 (D0)	41	51.8	(27.4; 97.9)	18	68.3	(28.7; 163)
	V04 (D28)	41	288	(163; 510)	18	74.8	(27.8; 201)
	Ratio V01 (D0) /PD3	41	0.403	(0.227; 0.714)	18	0.493	(0.256; 0.950)
	Ratio V04 (D28) /PD3	41	2.24	(1.30; 3.88)	18	0.540	(0.240; 1.22)
	Ratio V04 (D28) /V01 (D0)	41	4.55	(2.86; 7.22)	18	0.976	(0.556; 1.71)
Serotype 4 [PRNT - 1/dil]	Post dose 3 in CYD13 and CYD30 (PD3)	41	103	(65.9; 162)	18	119	(71.9; 197)
	V01 (D0)	41	54.9	(37.2; 80.9)	18	31.2	(16.1; 60.3)
	V04 (D28)	41	347	(183; 657)	18	37.0	(17.9; 76.6)
	Ratio V01 (D0) /PD3	41	0.487	(0.278; 0.854)	18	0.252	(0.133; 0.476)
	Ratio V04 (D28) /PD3	41	3.08	(1.50; 6.36)	18	0.299	(0.148; 0.604)
	Ratio V04 (D28) /V01 (D0)	41	5.91	(2.99; 11.7)	18	0.980	(0.632; 1.52)

TABLE 7B – NAb titres against each serotype at PD3, pre-booster injection and 28 days post-booster injection in subjects dengue immune at baseline (i.e. at D0 in the previous trials CYD13 and CYD30)

		CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)		
Component	Time point/ratio	M	GM	(95% CI)	M	GM	(95% CI)
Serotype 1 [PRNT - 1/dil]	Post dose 3 in CYD13 and CYD30 (PD3)	136	656	(501; 861)	46	463	(278; 771)
	V01 (D0)	136	668	(498; 895)	46	725	(413; 1273)
	V04 (D28)	136	940	(723; 1222)	46	650	(358; 1181)
	Ratio V01 (D0) /PD3	136	1.00	(0.803; 1.25)	46	1.52	(0.913; 2.53)
	Ratio V04 (D28) /PD3	136	1.41	(1.15; 1.73)	46	1.36	(0.840; 2.21)
	Ratio V04 (D28) /V01 (D0)	136	1.38	(1.16; 1.65)	46	0.882	(0.668; 1.17)
Serotype 2 [PRNT - 1/dil]	Post dose 3 in CYD13 and CYD30 (PD3)	135	613	(474; 792)	46	511	(365; 713)
	V01 (D0)	136	657	(505; 853)	46	647	(408; 1025)
	V04 (D28)	136	922	(734; 1158)	46	705	(439; 1132)
	Ratio V01 (D0) /PD3	135	1.07	(0.827; 1.38)	46	1.27	(0.794; 2.02)
	Ratio V04 (D28) /PD3	135	1.50	(1.17; 1.94)	46	1.38	(0.871; 2.19)
	Ratio V04 (D28) /V01 (D0)	136	1.38	(1.16; 1.65)	46	1.09	(0.782; 1.52)
Serotype 3 [PRNT - 1/dil]	Post dose 3 in CYD13 and CYD30 (PD3)	134	1045	(849; 1286)	46	921	(627; 1353)
	V01 (D0)	136	638	(502; 811)	46	918	(581; 1452)
	V04 (D28)	136	866	(689; 1089)	46	857	(561; 1311)
	Ratio V01 (D0) /PD3	134	0.585	(0.483; 0.710)	46	0.997	(0.655; 1.52)
	Ratio V04 (D28) /PD3	134	0.817	(0.668; 0.999)	46	0.931	(0.617; 1.40)
	Ratio V04 (D28) /V01 (D0)	136	1.34	(1.12; 1.61)	46	0.934	(0.724; 1.20)
Serotype 4 [PRNT - 1/dil]	Post dose 3 in CYD13 and CYD30 (PD3)	135	315	(249; 400)	45	346	(253; 475)
	V01 (D0)	136	224	(186; 269)	46	307	(214; 441)
	V04 (D28)	136	343	(281; 418)	46	287	(205; 402)
	Ratio V01 (D0) /PD3	135	0.664	(0.527; 0.838)	45	0.876	(0.584; 1.31)
	Ratio V04 (D28) /PD3	135	1.03	(0.786; 1.34)	45	0.825	(0.551; 1.24)
	Ratio V04 (D28) /V01 (D0)	136	1.52	(1.25; 1.86)	46	0.933	(0.744; 1.17)

Table 8. Seroconversion rate against each serotype in dengue immune and non-immune subjects

	Seroconversion Rate	
Serotype	Dengue Immune	Dengue Non-Immune
1	10.3% (95% CI: 5.7; 16.7)	39.0% (95% CI: 24.2; 55.5)
2	13.2% (95% CI: 8.0; 20.1)	39.0% (95% CI: 24.2; 55.5)
3	11.0% (95% CI: 6.3; 17.5)	51.2% (95% CI: 35.1; 67.1)
4	15.4% (95% CI: 9.8; 22.6)	34.1% (95% CI: 20.1; 50.6)

Table 9. Safety overview after booster injection.

	CYD Dengue Vaccine Group (N=187)			**Placebo Group (N=64)**		
Subjects experiencing at least one:	**n/M**	**%**	**(95% CI)**	**n/M**	**%**	**(95% CI)**
Within 30 minutes after booster injection						
Immediate unsolicited AE	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
Immediate unsolicited AR	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
Within 28 days after booster injection						
Solicited reaction	114/187	61.0	(53.6; 68.0)	31/64	48.4	(35.8; 61.3)
Solicited injection site reaction	47/187	25.1	(19.1; 32.0)	12/64	18.8	(10.1; 30.5)
Solicited systemic reaction	105/187	56.1	(48.7; 63.4)	28/64	43.8	(31.4; 56.7)
Unsolicited AE	48/187	25.7	(19.6; 32.6)	13/64	20.3	(11.3; 32.2)
Unsolicited AR	2/187	1.1	(0.1; 3.8)	0/64	0.0	(0.0; 5.6)
Unsolicited non-serious AE	48/187	25.7	(19.6; 32.6)	13/64	20.3	(11.3; 32.2)
Unsolicited non-serious AR	2/187	1.1	(0.1; 3.8)	0/64	0.0	(0.0; 5.6)
Unsolicited non-serious injection site AR	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
Unsolicited non-serious systemic AE	48/187	25.7	(19.6; 32.6)	13/64	20.3	(11.3; 32.2)
Unsolicited non-serious systemic AR	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
AE leading to study discontinuation†	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
SAE	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
Death	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
Serious AESI	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
Non-serious AESI	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
During the study						
SAE	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
Death	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
Serious AESI	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)

n: number of subjects experiencing the endpoint listed in the first column

M: number of subjects with available data for the relevant endpoint

† Identified in the termination form as SAE or other AE or in an AE form that was at least Grade 1 and was within the time period indicated

Overall, 61.0% of subjects in the CYD-TDV Dengue Vaccine Group and 48.4% in the Placebo Group experienced at least 1 solicited reaction after the booster injection. Among these subjects, 8.0% of subjects in the CYD-TDV Dengue Vaccine Group and 6.3% in the Placebo Group reported at least 1 Grade 3 solicited reaction. The Grade 3 solicited reactions that were reported in each group were mostly systemic reactions. Following the booster injection, the 2 treatment groups were comparable in terms of number, intensity, time of onset, and duration of solicited reactions. The most frequently reported solicited injection site reaction in both groups was injection site pain (24.6% in the CYD-TDV Dengue Vaccine Group and 18.8% in the Placebo Group). One (0.5%) subject experienced an injection site erythema (in the CYD-TDV Dengue Vaccine Group), and no injection site swelling was reported in either group. Most solicited injection site reactions reported were of Grade 1 intensity, occurred within 3 days, and resolved spontaneously within 3 days. One (0.5%) subject in the CYD-TDV Dengue Vaccine Group reported a Grade 3 reaction (injection site pain). The most frequently reported solicited systemic reaction in both groups was headache. At least 1 episode of headache was reported in 46.5% of subjects in the CYD-TDV Dengue Vaccine Group and in 34.4% of subjects in the Placebo Group after injection. The proportions of subjects who reported at least 1 episode of myalgia, malaise, and asthenia were within the same range and were similar across treatment groups (between 21% and 32%). Some 7.9% of subjects in the CYD-TDV Dengue Vaccine Group and 9.5% of subjects in the Placebo Group experienced at least 1 episode of fever. A total of 15 (8.0%) subjects in the CYD-TDV Dengue Vaccine Group and 4 (6.3%) subjects in the Placebo Group experienced at least 1 Grade 3 solicited systematic reaction. Headache was the most frequent Grade 3 systemic reaction, it was reported by 11 (5.9%) subjects from the CYD-TDV Dengue Vaccine Group and by 2 (3.1%) subjects in the Placebo Group. In the CYD-TDV Dengue Vaccine Group, one (0.5%) subject experienced at least 1 immediate unsolicited non-serious AR. The subject experienced a Grade 2 lump in the right axilla. This systemic event spontaneously resolved after 5 days and was assessed as related to the booster injection by the Investigator. Few unsolicited non-serious AEs reported within 28 days after injection were related to vaccination by the Investigator. One subject in the CYD-TDV Dengue Vaccine Group experienced an immediate unsolicited systemic AR (Grade 2 lump in the right axilla). A second subject in the same Group experienced 1 unsolicited non-serious AR (Grade 1 muscular weakness). For both subjects, the ARs occurred within 3 days after booster injection, spontaneously resolved within 4-7 days. Five SAE had been reported, it was considered as not related to vaccination. No AEs considered as significant (i.e., AEs and SAEs leading to discontinuation, AESIs, and hospitalized VCD cases) and None deaths were reported within 28 days after booster injection.

Long Term Follow Up

Persistence of neutralizing antibodies at six months and 1 year post-booster/placebo dose was measured by PRNT in available subjects (both dengue naïve and dengue immune at baseline) and the results are shown by serotype in Tables 10 to 14. As has been seen with other long term follow up analyses of recipients of CYD dengue vaccine, there was a decline in neutralizing antibody titres in the six month period following the administration of Dengvaxia® (booster dose), but after that point, neutralizing antibody titres stabilized, at least until 1 year post booster. Surprisingly, in subjects who were dengue naïve at baseline, the relative rate of decline was lower than in subjects who were dengue immune at baseline. For example, in immune subjects, the GMT levels at 12 months post booster dose for all four serotypes had already fallen below the GMT levels measured just before administration of the booster dose (i.e. at V01/D0). However, in naïve subjects, the GMT levels at 12 months post booster dose for all four serotypes were higher than the GMT levels measured just before administration of the booster dose (i.e. at V01/D0). This result is most easily seen in Table 15, which shows the M12:D0 GMT ratios for each serotype in both baseline naïve and baseline immune subjects. Thus it can be seen that the additive effect of the booster dose in baseline naïve subjects is surprisingly more durable in baseline naïve subjects than in baseline immune patients..

Table 11 : **Summary of Geometric Mean Titers of antibodies against each serotype with the parental dengue virus strains by baseline dengue status in CYD13/CYD30-Dengue PRNT-Per Protocol Analysis Set. SEROTYPE 1**

Time point	SEROTYPE 1 [PRNT-1/dil]											
	IMMUNE						NAIVE					
	CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)			CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)		
	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)
Post dose 3 in CYD13 and CYD30 (PD3)	136	656	(501;861)	46	463	(278; 771)	40	26.2	(17.0; 40.4)	18	39.9	(19.3;82.4)
V01 (D0)	136	668	(498; 895)	46	725	(413;1273)	41	29.6	(15.6; 56.1)	19	54.0	(21.4; 136)
V04 (D28)	**136**	**940**	**(723; 1222)**	**46**	**650**	**(358; 1181)**	**41**	**100**	**(52.5; 192)**	**18**	**40.4**	**(12.6; 130)**
V05 (M6)	**134**	**469**	**(362; 607)**	**45**	**396**	**(237; 663)**	**40**	**42.4**	**(25.1; 71.6)**	**18**	**31.2**	**(11.0; 88.2)**
V06 (M12)	**132**	**473**	**(366;611)**	**46**	**351**	**(221; 559)**	**38**	**38.6**	**(23.0; 64.6)**	**16**	**29.2**	**(11.0; 77.9)**

M : number of subjects available for the endpoint GM: Geometric Mean

V01 (D0): pre-booster or placebo injection V04 (D28): 28 days post booster or placebo injection

V05 (M6): 6 months post booster or placebo injection V06 (M12): 12 months post booster or placebo injection

Table 12 : Summary of Geometric Mean Titers of antibodies against each serotype with the parental dengue virus strains by baseline dengue status in CYD13/CYD30-Dengue PRNT-Per Protocol Analysis Set. SEROTYPE 2

Time point	SEROTYPE 2 [PRNT-1/dil]											
	IMMUNE						NAIVE					
	CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)			CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)		
	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)
Post dose 3 in CYD13 and CYD30 (PD3)	135	613	(474; 792)	46	511	(365; 713)	40	57.1	(39.6; 82.3)	18	79.9	(39.3; 163)
V01 (D0)	136	657	(505; 853)	46	647	(408; 1025)	41	48.9	(25.4; 94.1)	18	55.0	(20.2; 150)
V04 (D28)	**136**	**922**	**(734; 1158)**	46	**705**	**(439; 1132)**	**41**	**213**	**(121; 375)**	**18**	**61.0**	**(17.5; 213)**
V05 (M6)	**134**	**609**	**(500; 741)**	45	**617**	**(435; 873)**	**40**	**151**	**(89.5; 256)**	**18**	**72.6**	**(27.7; 191)**
V06 (M12)	**132**	**398**	**(327; 485)**	46	**388**	**(288; 523)**	**38**	**69.4**	**(42.3; 114)**	**16**	**47.2**	**(18.1; 123)**

M : number of subjects available for the endpoint GM: Geometric Mean

V01 (D0): pre-booster or placebo injection V04 (D28): 28 days post booster or placebo injection

V05 (M6): 6 months post booster or placebo injection V06 (M12): 12 months post booster or placebo injection

Table 13: Summary of Geometric Mean Titers of antibodies against each serotype with the parental dengue virus strains by baseline dengue status in CYD13/CYD30-Dengue PRNT-Per Protocol Analysis Set. SEROTYPE 3

Time point	SEROTYPE 3 [PRNT-1/dil]											
	IMMUNE						NAIVE					
	CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)			CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)		
	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)
Post dose 3 in CYD13 and CYD30 (PD3)	134	1045	(849; 1286)	46	921	(627; 1353)	41	129	(98.1; 168)	18	138	(82.0; 234)
V01 (D0)	136	638	(502; 811)	46	918	(581; 1452)	41	51.8	(27.4; 97.9)	18	68.3	(28.7; 163)
V04 (D28)	**136**	**866**	**(689; 1089)**	46	**857**	**(561; 1311)**	**41**	**288**	**(163; 510)**	**18**	**74.8**	**(27.8; 201)**
V05 (M6)	**134**	**748**	**(596; 939)**	45	**776**	**(503; 1197)**	**40**	**132**	**(79.5; 221)**	**18**	**113**	**(44.5; 284)**
V06 (M12)	**132**	**433**	**(347; 540)**	46	**528**	**(362; 769)**	**38**	**88.0**	**(54.5; 142)**	**16**	**83.4**	**(34.4; 202)**

M : number of subjects available for the endpoint GM: Geometric Mean

V01 (D0): pre-booster or placebo injection V04 (D28): 28 days post booster or placebo injection

V05 (M6): 6 months post booster or placebo injection V06 (M12): 12 months post booster or placebo injection

Table 14: Summary of Geometric Mean Titers of antibodies against each serotype with the parental dengue virus strains by baseline dengue status in CYD13/CYD30-Dengue PRNT-Per Protocol Analysis Set. SEROTYPE 4

Time point	SEROTYPE 4 [PRNT-1/dil]											
	IMMUNE						NAIVE					
	CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)			CYD Dengue Vaccine Group (N=177)			Placebo Group (N=64)		
	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)
Post dose 3 in CYD13 and CYD30 (PD3)	135	315	(249; 400)	45	346	(253; 475)	41	103	(65.9; 162)	18	119	(71.9; 197)
V01 (D0)	136	224	(186; 269)	46	307	(214; 441)	41	54.9	(37.2; 80.9)	18	31.2	(16.1; 60.3)
V04 (D28)	**136**	**343**	**(281; 418)**	46	**287**	**(205; 402)**	**41**	**347**	**(183; 657)**	**18**	**37.0**	**(17.9; 76.6)**
V05 (M6)	**134**	**249**	**(213; 292)**	45	**247**	**(193; 315)**	**40**	**185**	**(117; 295)**	**18**	**49.9**	**(26.2; 95.0)**
V06 (M12)	**132**	**192**	**(164; 225)**	46	**193**	**(157; 238)**	**38**	**110**	**(69.1; 175)**	**16**	**37.8**	**(20.9; 68.3)**

M : number of subjects available for the endpoint

GM: Geometric Mean

V01 (D0): pre-booster or placebo injection

V04 (D28): 28 days post booster or placebo injection

V05 (M6): 6 months post booster or placebo injection

V06 (M12): 12 months post booster or placebo injection

Table 15 – M12:D0 GMT ratios for each serotype in baseline naïve and baseline immune subjects

	Baseline naïve	Baseline immune
Serotype 1	1.30	0.71
Serotype 2	1.42	0.61
Serotype 3	1.70	0.68
Serotype 4	2.00	0.85

Conclusions:

- The primary objective of the CYD64 study is met. In subjects having received a 3-dose primary series of the CYD dengue vaccine 4-5 years before, the booster dose is non-inferior to the third dose, in terms of GMTRs.
- In subjects having received a 3-dose primary series of the CYD dengue vaccine 4-5 years before, the booster dose is not superior to the third dose of the primary series, in terms of GMTRs. Overall superiority is not attained as individual serotypes' superiority is not demonstrated for serotype 3.
- The CYD dengue vaccine booster increases GMTs of each serotype 28 days after injection.
- The CYD dengue vaccine booster increases seropositivity rates against each and any serotypes.
- The dengue serostatus at baseline influences both the persistence of GMTs at pre-booster injection and the level of GMTs post-booster injection; i.e., subjects that were dengue-immune at baseline tended to have higher GMTs both at pre- and post-booster injection.
- **At 28 days after injection, dengue non-immune subjects at baseline have a higher seroconversion rate for each serotype than dengue-immune subjects at baseline; i.e., the increases of GMTs between pre-booster and post-booster injection are greater in subjects that were dengue non-immune (i.e. dengue naïve) at baseline compared to dengue immune subjects at baseline. This difference between dengue immune and dengue non-immune subjects at baseline is also demonstrated in the GMT ratios comparing the GMTs 28 days post booster compared to the GMTs PD3 of the primary vaccination course.**
- **The additive effect of the booster dose in baseline naïve subjects is more durable in baseline naïve subjects than in baseline immune patients.**

- A booster injection administered 4-5 years after a 3-dose primary schedule is quite similar, in terms of reactogenicity, to the first CYD dengue vaccine injection administered in CYD13 and CYD30.

Sequences referred to in this application:

Table 16: Sequences of the Sequence Listing

SEQ ID NO.	Sequence
1	prM-E nucleotide sequence of the serotype 1 vaccinal strain which is derived from the PUO 359 (TVP-1140) wild type strain
2	prM-E nucleotide sequence of the serotype 2 vaccinal strain which is derived from the PUO 218 wild type strain
3	prM-E nucleotide sequence of the serotype 3 vaccinal strain which is derived from the PaH881/88 wild type strain
4	prM-E nucleotide sequence of the serotype 4 vaccinal strain which is derived from the 1228 (TVP 980) wild type strain
5	prM-E nucleotide sequence of the serotype 2 vaccinal strain derived from the MD1280 wild type strain (CYD-2V)
6	Entire nucleotide sequence of the VDV1 strain
7	Entire nucleotide sequence of the VDV2 strain

The above listed nucleotide sequences constitute the positive strand RNA of the listed dengue viruses (i.e. the nucleotide sequence which is found in the corresponding viral particles). The equivalent DNA sequences (which may be used to manipulate and express the corresponding virus and which also form part of the disclosure of the present application), can be generated by replacing the nucleotide U with the nucleotide T. Such DNA sequences constitute the cDNA sequences of the corresponding dengue viruses.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of booster vaccination for inducing in a human subject a neutralizing antibody response against dengue virus, said method comprising administering to the subject a vaccine composition comprising a dengue antigen of each of serotypes 1 to 4 and wherein each of said dengue antigens is independently selected from the list consisting of: (a) a live attenuated dengue virus and (b) a live attenuated chimeric dengue virus, wherein said subject has previously received a primary vaccination course against each of serotypes 1 to 4 of dengue virus, and said subject was dengue naïve before said primary vaccination course, and wherein said vaccination composition is administered at least one year after the end of the primary vaccination course.

2. The method according to claim 1, wherein the method results in at least a 2-fold increase in the neutralizing antibody titre against each of serotypes 1 to 4.

3. The method according to claim 1 or claim 2, wherein the booster immunization is administered at least two years after the end of the primary vaccination course.

4. The method according to any one of claims 1 to 3, wherein said primary vaccination course is administered in one, two or three doses.

5. The method according to any one of claims 1 to 4, wherein said subject is at least 11 or 12 years old.

6. The method according to any one of claims 1 to 5, wherein the subject has, before booster administration, a neutralizing antibody titer against each of serotypes 1 to 4 of at least 10 and less than 150, preferably less than 120.

7. The method according to any one of claims 1 to 6, wherein said 2-fold increase in the neutralizing antibody titre against each of serotypes 1 to 4 is measured between 20 and 60 days after said booster vaccination, preferably 28 days after said booster vaccination.

8. The method according to any one of claims 1 to 7, wherein said neutralizing antibody titre is measured using a dengue Plaque Reduction Neutralization Test ($PRNT_{50}$) test.

9. The method according to any one of claims 1 to 8, wherein said vaccine composition administered for booster vaccination is identical to the vaccine composition previously administered during the primary vaccination course.

10. The method according to any one of claims 1 to 8, wherein said vaccine composition administered for booster vaccination is different from the vaccine composition previously administered during the primary vaccination course.

11. The method according to any one of claims 1 to 10, wherein said subject has not previously been naturally infected by a dengue virus.

12. The method according to any one of claims 1 to 11, wherein said dengue antigens of serotypes 1 to 4 comprise a nucleic acid sequence having at least 90% identity to SEQ ID NOs: 1, 2, 3 and 4 respectively.

13. The method according to any one of claims 1 to 12, wherein the amino acid at position 226 of the Envelope (E) protein of the dengue antigen of serotype 2 is threonine, the amino acid at position 228 of the Envelope (E) protein of the dengue antigen serotype 2 is glycine, and the amino acid at position 251 of the Envelope (E) protein of the dengue antigen of serotype 2 is valine.

14. A method of inducing in a human subject a neutralizing antibody response against dengue virus, comprising administering to the subject a vaccine composition comprising a dengue antigen of each of serotypes 1 to 4, wherein said dengue antigens of serotypes 1 to 4 are each independently selected from the group consisting of a live attenuated dengue virus and a live attenuated chimeric dengue virus;
wherein said vaccine composition is administered as:
    (a) a primary vaccination, followed at least 1 year after the end of the primary vaccination course by
    (b) a booster vaccination,
and wherein the human subject is initially dengue naive.

15. The method according to claim 14, wherein the booster immunization is administered at least two years after the end of the primary vaccination course.

16. The method according to claim 14 or claim 15, wherein said primary vaccination course is administered in one, two or three doses.

17. The method according to any one of claims 14 to 16, wherein said subject is at least around 9 years of age.

18. The method according to any one of claims 14 to 17, wherein said booster vaccination results in at least a 2-fold increase in the neutralizing antibody titre against each of serotypes 1 to 4, when compared with the neutralizing antibody titres induced after the primary vaccination.

19. The method according to claim 18, wherein said 2-fold increase in the neutralizing antibody titre against each of serotypes 1 to 4 is measured between 20 and 60 days after said booster vaccination, preferably 28 days after said booster vaccination.

20. The method according to claim 18 or 19, wherein said neutralizing antibody titre is measured using a dengue Plaque Reduction Neutralization Test ($PRNT_{50}$) test.

21. The method according to any one of claims 1 to 20, wherein said human subject is protected against dengue disease, preferably against severe dengue disease.

22. The method according to any one of claims 1 to 21, wherein said subject is aged between 9 months and 60 years old.

23. The method according to claim 14, wherein said primary vaccination course consists in administration of 3 vaccine doses, wherein the second dose is administered about 6 months after the first dose and the third dose is administered about 6 months after the second dose.

24. The method according to any one of claims 1 to 23, wherein the booster immunization is administered less than 20 years after the end of the primary vaccination course, preferably less than 10 years.

25. The method according to any one of claims 1 to 24, wherein the human subject is resident in a dengue endemic area.

26. The method according to any one of claims 1 to 25, wherein said composition comprises a dengue antigen of each of serotypes 1 to 4 and wherein each of said dengue antigens is a live attenuated chimeric dengue virus.

27. The method according to any one of claims 1 to 26, wherein said live attenuated chimeric dengue virus comprises a genome from a first flavivirus in which the prM-E sequence has been replaced with a prM-E sequence of a dengue virus.

28. The method according to claim 27, wherein said first flavivirus is a yellow fever virus or a dengue virus, preferably a yellow fever virus.

29. The method according to any one of claims 14 to 28, wherein said dengue antigens of serotypes 1 to 4 comprise a nucleic acid sequence having at least 90% identity to SEQ ID NOs: 1, 2, 3 and 4 respectively.

30. The method according to any one of claims 14 to 29, wherein the amino acid at position 226 of the Envelope (E) protein of the dengue antigen of serotype 2 is threonine, the amino acid at position 228 of the Envelope (E) protein of the dengue antigen serotype 2 is glycine, and the amino acid at position 251 of the Envelope (E) protein of the dengue antigen of serotype 2 is valine.

31. Kit when used in a method as defined in any one of claims 1 to 30, the kit comprising a vaccine composition as defined in claim 1 or claim 14 and instructions for the use of said vaccine composition when used in a method as defined in any one of claims 1 to 30.

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<151> 2017-10-05

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