

**A DENGUE VIRUS CHIMERIC POLYPEPTIDE COMPOSED OF
FRAGMENTS OF NON-STRUCTURAL PROTEINS AND ITS USE IN AN
IMMUNOGENIC COMPOSITION AGAINST DENGUE VIRUS INFECTION**

FIELD OF THE INVENTION

The present invention is directed to a dengue virus chimeric polypeptide composed of fragments of non-structural proteins and its use in an immunogenic composition against dengue virus infection. The present invention provides means, in particular polynucleotides, vectors, cells and methods to produce vectors expressing said chimeric polypeptides, in particular vectors consisting of recombinant measles virus (also designated MV) particles. The present invention also relates to the use of the recombinant MV particles, in particular under the form of a composition or of a vaccine, for the prevention and/or treatment of a dengue virus infection.

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BACKGROUND OF THE INVENTION

Dengue virus (DENV) belongs to the *Flaviviridae* family of enveloped, positive-strand RNA viruses, and is transmitted by *Aedes* mosquitoes. DENV infection is the most important arthropod-borne viral disease, with about 390 million infections every year, that can result in dengue fever (DF), and in 1-5% of cases in dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), characterized by vascular leak leading to hypotensive shock (*World Health Organization (WHO) Press 2009, WHO/HTM/NTD/DEN/2009. 1 ed.; Bhatt S et al., Nature 2013, 496(7446):504-507*). Over 2 million cases of severe dengue disease and over 20,000 deaths are estimated to occur each year (*Gubler DJ, The American journal of tropical medicine and hygiene 2012, 86(5):743-744*).

There are four main DENV serotypes (designated DENV1-4) that are 67-75% identical at the amino acid level. The viral RNA genome is translated as a single polyprotein that is cleaved by viral and host proteases into three

structural proteins (capsid (C), premembrane (prM), and envelope (E)) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). Complete nucleotide sequences of the reference genomes of the 4 dengue virus serotypes can be accessed from the Genbank database under
5 accession numbers NC_001477.1, NC_001474.2, NC_001475.2 and NC_002640.1 respectively. During infection, the E protein interacts with cellular receptors and viral uptake occurs via receptor-mediated endocytosis (*Heinz FX et al., Archives of virology Supplementum 1994, 9:339-348; Mukhopadhyay S et al., Nat Rev Microbiol 2005, 3(1):13-22*). The E protein is
10 structurally conserved among flaviviruses and consists of three domains (EDI, EDII, and EDIII) (*Rey FA et al., Nature 1995, 375(6529):291-298*). Notably, the EDIII domain induces the most potent neutralizing and serotype-specific antibodies (*Beltramello M et al., Cell host & microbe 2010, 8(3):271-283; Shrestha B et al., PLoS Pathog 2010, 6(4):e1000823; Sukupolvi-Petty S et al., J Virol 2010, 84(18):9227-9239; Wahala WM et al., PLoS Pathog 2010, 6(3):e1000821; de Alwis R et al, PLoS neglected tropical diseases 2011, 5(6):e1188; Yauch LE et al., Advances in virus research 2014, 88:315-372*).
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A single minimal tetravalent DENV antigen composed of the four envelope domain III (EDIII) from the four DENV serotypes fused to the
20 ectodomain of the membrane protein (ectoM) has been previously described (*Brandler et al., PLoS 2007, 1(3), e96; Brandler et al., Vaccine, 2010, 28, 6730-6739*). When expressed by a replicating viral vector derived from live-attenuated MV vaccine, this antigen induced neutralizing antibodies against the four serotypes of dengue virus (*Brandler et al., Vaccine, 2010, 28, 6730-6739*).
25 However, evaluated in a non-human primate model of DENV infection, a recombinant MV vector expressing the tetravalent EDIII-ectoM antigen provided only partial protection. This observation indicated that an additional DENV antigen was missing to provide full protection.

The NS proteins are involved in viral replication and assembly and are
30 usually not incorporated in mature viral particles. Strikingly, while a primary infection by one DENV serotype induces a lasting protective immunity

against reinfection by the same serotype, not only it does not protect against infection with other serotypes but it increases also the risk to develop a more severe disease upon secondary infection, a phenomenon attributed to non-neutralizing or sub-neutralizing antibodies and called Antibody-Dependent Enhancement (ADE) (*Halstead SB et al. Nature 1977, 265(5596):739-741; Dejnirattisai W et al. Science 2010, 328(5979):745-748*). In support of the ADE hypothesis, it was proposed that the low levels of serotype cross-reactive antibodies produced following a primary infection, can enhance the secondary infections through the formation of DENV-antibody complexes that bind to the Fcγ receptors (FcγR) on myeloid cells. This process leads to higher viral load and higher production of inflammatory mediators responsible of vascular permeability (*Halstead SB et al. Nature 1977, 265(5596):739-741; Morens DM et al., Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 1994, 19(3):500-512; Halstead SB et al., Advances in virus research 2003, 60:421-467*).

Mechanistically, ADE was shown to be critically dependent on the activation of FcγRIIIa receptor expressed by monocytes, macrophages and dendritic cells (DCs) which produce high amounts of cytokines (TNF-α and IL-6) and chemokines (MIP-1α) upon stimulation (*Wong KL et al., PLoS ONE 2012, 7(5):e36435; Boonnak K et al., J Immunol 2013, 190(11):5659-5665; Guilliams M et al., Nat Rev Immunol 2014, 14(2):94-108*). The increased viral loads in ADE were also shown to result from the binding of immune complexes (between the virus and sub-neutralizing antibodies) to the leukocyte Ig-like receptor B1 (LILR-B1) on human primary monocytes, leading to the inhibition of the early antiviral responses mediated by the activated FcγRIIIa (*Chan KR et al., Proc Natl Acad Sci USA 2014, 111(7):2722-2727*).

Thus, depending on the nature of the DENV-specific antibody response, the adaptive immunity can induce either protection against infection or enhancement of infection and disease progression.

Like B cells, a pathogenic role of virus-specific T cells during secondary infection was also suggested. The hypothesis, called “original antigenic sin” postulated that, after a primary infection, cross-reactive memory T cells, with low avidity for the serotype of the secondary infection, dominate and mask the specific T cell response, leading to a less efficient killing of infected target cells (*Mongkolsapaya J et al. Nat Med 2003, 9(7):921-927*). These cross-reactive CD8+ T cells, stimulated upon a secondary infection with a different serotype, displayed also quantitative and qualitative differences in their response to the cross-reactive epitope or the altered peptide ligand (*Bashyam HS et al., J Immunol 2006, 176(5):2817-2824*).

However, in spite of these studies, the direct demonstration of a pathogenic role of DENV-specific T cells is still missing, and recent reports did not support a causative role for cross-reactive CD8+ T cells in the pathogenesis of dengue hemorrhagic fever during secondary infections. Indeed, a study in adults experiencing a secondary infection did not reveal any correlation between the magnitude and specificity of T-cell responses and clinical disease grade (*Simmons CP et al., J Virol 2005, 79(9):5665-5675*), and an important protective role for CD8+ T cells during primary DENV infection was also identified in a mouse model (*Yauch LE et al. J Immunol 2009, 182(8):4865-4873*). More strikingly, a detailed analysis of HLA-restricted T-cell responses in donors from hyperendemic area even reinforces the protective role of CD8+ T cells during DENV infection (*Weiskopf D et al., Proc Natl Acad Sci USA 2013, 110(22):E2046-2053*). It appears that, whereas serotype-specific responses are a hallmark of primary infection, there is a shift towards a response against conserved epitopes following secondary infection, without any difference in the avidity or functionality in CD8+ T cells among serotype-specific or conserved responses. In addition, a significant correlation was established between a weak T-cell response and disease susceptibility (*Weiskopf D et al., Proc Natl Acad Sci USA 2013, 110(22):E2046-2053*). Collectively, these studies

highlight the beneficial effect of CD8+ T cells against disease progression in dengue virus infection.

BRIEF DESCRIPTION OF THE FIGURES

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Figure 1. Immunogenicity of non-structural proteins NS3, NS4a, NS4b and NS5 of DENV1 and intra-serotype and inter-serotype amino acid sequence conservation of DENV. A. Weiskopf D *et al.*, Proc Natl Acad Sci USA 2013, 110(22):E2046-2053; B. Rivino L *et al.*, J Virol 2013, 87(5):2693-2706.

Figure 2. Amino acid sequence and secondary structure of NS DENV1 polyepitopic combined polyepitopic antigen (SEQ ID NO: 3).

The following recitation of positions of amino acids in the sequence indicates the type of secondary structure in which the amino acid(s) is(are) involved in the corresponding full length protein as predicted for NS4B, or described in published crystallography studies for NS3 and NS5 (PDB ID 2VBC, *J Virol.* 2008 Jan;82(1):173-183 and 2J7W, *J Virol.* 2007 May;81(9):4753-65, respectively). In particular, **alpha-helices** are observed at the following segments of amino acid(s): 35-45 ; 57-66 ; 94-103 ; 123-138 ; 181-185 ; 189-198 ; 232-241 ; 247-254 ; 307-318 ; 342-360 ; 371-372 ; 374-375 ; 396-398 ; 406-419 ; 455-460 ; 468-473 ; 480-489 ; 500-517. **Beta strands** are observed at the following segments of amino acid(s): 20-23 ; 50-54 ; 72-74 ; 112-116 ; 142-146 ; 164-168 ; 276-277 ; 280-281 ; 283-285 ; 291-293 ; 366-370 ; 376-380 ; 436-442 ; 452-453. **Bends** are observed at the following segments of amino acid(s): 16 ; 55-56 ; 70 ; 80 ; 86-87 ; 89-93 ; 105 ; 111 ; 117 ; 122 ; 140 ; 148-49 ; 156-157 ; 161-162 ; 173-174 ; 178-179 ; 221 ; 243 ; 260-261 ; 287-288 ; 297-298 ; 305-306 ; 433-35 ; 449-450 ; 479 ; 492 ; 519-520. **Turns** are observed at the following segments of amino acid(s): 17-18 ; 27-29 ; 32-34 ; 46-47 ; 67-69 ; 77-78 ; 118-119 ; 139 ; 151-152 ; 186-187 ; 199-200 ;

204-205 ; 223-226 ; 230-231 ; 242 ; 255-257 ; 264-267 ; 278-279 ; 304 ;
 319 ; 329-332 ; 420-423 ; 428-429 ; 474 ; 490-491 ; 518 ; 528-538.
 Moreover, **3/10 helices** are observed at the following segments of amino
 acid(s): 213-218 ; 271-273 ; 301-303 ; 462-466 and **β bridge** is observed
 5 at the amino acids 188 and 300. Positions with conserved amino acids
 (found in more than 99.9% of a representative panel of 2033 sequences
 that include the 4 serotypes) are highlighted in dark grey.

Figure 3. Amino acid consensus sequences of DENV2-NS, DENV3-NS and
 10 DENV4-NS polyepitopes (SEQ ID NOs: 146, 147 and 148 respectively).

Figure 4. Alignment comparing the amino acid sequences of DENV1-NS,
 DENV2-NS, DENV3-NS and DENV4-NS consensus polyepitopes (SEQ ID
 NOs: 3, 146, 147 and 148 respectively).

15 **Figure 5.** Native nucleotide sequence of the polynucleotide encoding
 DENV1-NS polyepitope (SEQ ID NO: 1).

Figure 6. Optimised nucleotide sequence of the polynucleotide encoding
 20 DENV1-NS polyepitope (human codon optimized, measles optimized)
 (SEQ ID NO: 2).

**Figure 7. Schematic representation of recombinant MV vectors
 expressing DENV antigens.** The NS polyepitopic synthetic sequence
 25 was inserted into the ATU position 2 or 3 of MV vectors in combination or
 not with DENV EDIII tetrameric antigen. The MV genes are indicated as
 follows: nucleoprotein (N), phosphoprotein and V/C accessory proteins
 (PVC), matrix (M), fusion (F), hemagglutinin (H) and polymerase (L). T7
 RNA polymerase promoter (T7), T7 RNA polymerase terminator (T7t),
 30 hepatitis delta virus ribozyme (∂), hammerhead ribozyme (hh). In
 MVDVax9, the combined antigen inserted into the ATU position 2 is
 composed of a secreted DENV EDIII tetrameric protein fused to a

secreted NS polyepitopic sequence with furin sites (having the amino acid sequence RRDKR as defined in SEQ ID NO: 152) inserted to separate the different components. In MVDVax11, on the contrary, the NS polyepitopic synthetic sequence is not secreted (no peptide leader sequence) and is fused in 3' to the exported DENV EDIII tetrameric protein (starting with peptide leader sequence).

Figure 8. Detection by western blot of the expression of DENV NS polyepitopic antigen (top) and DENV EDIII tetrameric antigen (bottom) in cell lysates of Vero cells infected by MV-DENV recombinant viruses or HEK293 cells transfected with pcDNA3-NS plasmid. DENV proteins were probed with specific Mabs.

Figure 9. DENV NS polyepitope peptides used for ELISPOT assays in CD46-IFNAR mice. Sequences 1-36 are 15-mer peptides overlapping of 5 aminoacids covering the entire DENV NS polyepitopic antigen. Peptides 37-41 were identified using prediction algorithms for their ability to bind H2-Db and H2-Kb T cell receptors expressed in CD46-IFNAR mice. These peptides have amino acid sequences as defined in SEQ ID NOs: 98-138.

Figure 10. ELISPOT quantification of T-cell responses in CD46-IFNAR mice immunized by MV-DENV vectors. Groups of six mice were immunized with MVDVax10, MVDVax8 or empty MV. ELISPOTS were quantified in splenocytes collected 7 days after a single immunization. Responses to DENV NS peptides, MV and concanavalin A as a positive control are shown.

Figure 11. ELISPOT quantification of T-cell responses in CD46-IFNAR mice immunized by MV-DENV vectors. The responses to different peptide pools or individual peptides covering the NS polyepitopic antigen are shown.

Figure 12. ELISPOT responses in CD46-IFNAR mice immunized by MV-DEBNV vectors cumulating the responses to individual peptides or pools.

- 5 **Figure 13. Procedure for eliciting CD8+ T cell responses in H-2 Class I null/ HLA Class I transgenic mice and quantification of IFN- γ response by ELISPOT assay.**

- 10 **Figure 14. Identification and HLA restriction of the immunogenic epitopes of the DENV1-NS polyepitopic construct (SEQ ID NO: 3).**

- Figure 15. Quantification of the T-cell responses in HLA-A2, A24, B7 and B35 transgenic mice by ELISPOT assay.** Three independent experiments have been performed, in which a total of ten and four HLA-A*02:01 transgenic mice received the DENV1-NS polyepitopic construct (SEQ ID NO: 3) and the control plasmid, respectively, eight and four HLA-A*24:02 transgenic mice received the DENV1-NS polyepitopic construct and the control plasmid, respectively, five and four HLA-B*07:02 received the DENV1-NS polyepitopic construct and the control plasmid, respectively and five and three HLA-B*35:01 transgenic mice received the DENV1-NS polyepitopic construct and the control plasmid, respectively. All the animals were immunized by intradermic injection (100 μ g DENV1-NS polyepitopic construct, or 100 μ g pcDNA3.1 control plasmid) followed by *in vivo* electroporation. Two immunizations were performed at 3 week interval, and spleen cells were tested for IFN- γ secretion by ELISPOT 10 days after the second injection. Individual mice were tested in parallel with different peptides at 2 μ g/ml and with concanavalin A (ConA) at 5 μ g/ml, final concentration.

- 30 **Figure 16. A) IFN- γ and B) Granzyme B (GrB) ELISPOT responses cumulating the responses to individual peptides.** In these assays, the

peptides used were: For HLA-A24 transgenic mice, peptides P17, P32 and P33 (having the amino acid sequences as defined in SEQ ID NO: 39, 33 and 47 respectively) were used as cognate, and peptide HBSP A2-2 (having the amino acid sequence TLCIPHVAV as defined in SEQ ID NO: 150) as control.

5 For HLA-A2 transgenic mice, peptides P30, P53 and P56 were used as cognate (having the amino acid sequences as defined in SEQ ID NO: 18, 51 and 55 respectively), and peptide HBSP A2-2 as control. For HLA-B7 transgenic mice, peptides P15, P30 and P36 were used as cognate (having the amino acid sequences as defined in SEQ ID NO: 68, 18 and 21

10 respectively), and peptide Sv B7-3 (having the amino acid sequence SPFLPLLPI as defined in SEQ ID NO: 151) as control. For HLA-B35 transgenic mice, peptides P49, P50 and P51 were used as cognate (having the amino acid sequences as defined in SEQ ID NO: 28, 32 and 45 respectively), and peptide Sv B7-3 as control.

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Figure 17. *In vivo* cytotoxic activity of T cells from animals immunized with the DENV1-NS polyepitopic construct (SEQ ID NO: 3) or the control plasmid. For HLA-A24 mice, high stained target cells were pulsed with a mix of P17, P32 and P33 cognate peptides (having the amino acid sequences as defined in SEQ ID NO: 39, 33 and 47 respectively), while low stained control cells were not pulsed. For HLA-A2 mice, high stained target cells were pulsed with a mix of P30, P53 and P56 cognate peptides (having the amino acid sequences as defined in SEQ ID NO: 18, 51 and 55 respectively), while low stained control cells were pulsed with the HBSP A2-2 control peptide. For

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25 HLA-B35 mice, high stained target cells were pulsed with a mix of P49, P50 and P51 cognate peptides (having the amino acid sequences as defined in SEQ ID NO: 28, 32 and 45 respectively), while low stained control cells were not pulsed.

30 **DESCRIPTION OF THE INVENTION**

The inventors have analyzed transcripts differentially expressed in peripheral blood mononuclear cells (PBMCs) from either asymptomatic or symptomatic infected donors from a cohort in Cambodia and observed a significant number of genes corresponding to CD8⁺ T cell activation overexpressed in asymptomatic individuals, in comparison with symptomatic donors, with Dengue Fever (DF) or Dengue Hemorrhagic Fever (DHF). A higher number of transcripts associated with TH17 and neutrophil activation were also observed in PBMC from these symptomatic patients with DHF and dengue shock syndrome (DSS), in agreement with the strong inflammatory response observed in severe dengue cases. Taken together, these observations strongly support an HLA-linked protective role for CD8⁺ T cells in anti-DENV immunity.

The inventors designed an antigen capable of inducing CD8⁺ T cell immunity to DENV. Most importantly, this antigen has a small size compared to the full length of the DENV genome, or to the full length of DENV NS proteins, so that it can be inserted in any vaccine vector, such as for example, a replicating viral vector derived from live-attenuated MV vaccine. Furthermore, this design aims at providing a DENV antigen enriched in highly antigenic epitopes, with the idea of limiting the exposure of the immune system to weakly immunogenic epitopes, and prevent the dispersion or dilution of the immune response. This antigen is composed of polyepitopic regions from NS proteins of DENV. Using compiled anti-DENV T-cell epitope distribution and strength (*Simmons CP et al., J Virol 2005, 79(9):5665-5675; Yauch LE et al., J Immunol 2009, 182(8):4865-4873; Weiskopf D et al., Proc Natl Acad Sci USA 2013, 110(22):E2046-2053; Imrie A et al., J Virol 2007, 81(18):10081-10091; Lund O et al., PLoS ONE 2011, 6(10):e26494; Weiskopf D et al., J Immunol 2011, 187(8):4268-4279; Boucherma R et al., J Immunol 2013, 191(2):583-593; Nascimento EJ et al., PLoS neglected tropical diseases 2013, 7(10):e2497; Rivino L et al., J Virol 2013, 87(5):2693-2706; Rivino L et al., J Immunol 2013, 191(8):4010-4019; Immune Epitope Database (IEDB)), the inventors selected four regions that are enriched in*

CD8 epitopes, for a total of 539 amino acids: two regions in NS3 (185 and 134 amino acids, respectively), one region in NS4b (86 amino acids) and one in NS5 (135 amino acids) (**Figure 1**). Borders of these 4 regions were chosen based on the secondary structure and amino acid sequence properties of the respective polyproteins, using crystallographic data for NS3 and NS5 (PDB ID 2VBC and 2J7W respectively), and using prediction tools such as JPRED (Cole C, Barber JD & Barton GJ. *Nucleic Acids Res.* 2008. 35 (suppl. 2) W197-W201) or GOR IV (GOR secondary structure prediction method version IV, *Methods in Enzymology* 1996 R.F. Doolittle Ed., vol 266, 540-553, Garnier J, Gibrat J-F, Robson B) for NS4B, (**Figure 2**). Notably, the design carefully avoided the disruption of secondary structure elements such as α -helices or β -strands (**Figure 2**). A total of 2033 full-length dengue genome sequences (865 for serotype 1, 678 for serotype 2, 427 for serotype 3 and 63 for serotype 4) were aligned using MUSCLE 3.7 (*Edgar RC, Nucleic Acids Res* 2004, 32(5):1792-1797), with manual adjustments according to the amino acid sequence. Sequence similarity was evaluated intra- and inter-serotypes at the nucleic and amino acid levels. Analysis of the concatenated regions of interest revealed strong intra-serotype conservation, and generally a higher degree of sequence identity compared to the genome as a whole, including for inter subtypes comparisons (**Figures 1 and 2**). Based on the genetic diversity of the four serotypes of DENV, and with the idea that T cell epitopes are either conserved among different serotypes, or are serotype-specific with nevertheless the ability to induce cross-reactive CD8⁺ T-cell responses, a prototype consensus sequence based on epidemic strains of DENV1 was selected, the DENV1 NS T cell polyepitope (**Figures 1 and 2**).

A serotype 1 consensus sequence was selected, as it presented the highest average genetic identity with the 4 serotypes (versus serotype 1: 99.48%; serotype 2: 83.48%; serotype 3: 89.39%; serotype 4: 76.96%).

Average genetic identity was estimated for each serotype as the percent sequence identity (ratio of identical positions over the total of aligned positions constituting the NS polyepitope) pairwise for each sequence of the

serotype, and the average was reported. This percentage given corresponds only to the concatenation of the selected fragments of NS3, NS4B and NS5.

The invention thus relates to a chimeric polyepitope having less than 600 amino acid residues comprising or consisting of the following fragments
5 of (a), (b) and (c) assembled in a fusion polypeptide wherein the fragments of (a), (b) and (c) are directly or indirectly fused in this order:

(a) two fragments of the non-structural (NS) NS3 protein of the dengue virus (DENV) serotype 1 (DENV1) comprising or consisting of two regions, wherein the first region has an amino acid sequence as defined in SEQ ID
10 NO: 6, and the second region has an amino acid sequence as defined in SEQ ID NO: 9,

(b) a fragment of the NS4b protein of DENV1 having an amino acid sequence as defined in SEQ ID NO: 12,

(c) a fragment of the NS5 protein of DENV1 having an amino acid
15 sequence as defined in SEQ ID NO: 15,
or a polyepitope variant thereof, which (i) comprises or consists of the assembly in a fusion polypeptide, of DENV NS fragments, the sequences of which are obtained by alignment of the NS3 DENV1, NS4b DENV1, NS5 DENV1 fragments recited in (a), (b) and (c) with the respective NS3, NS4b
20 and NS5 sequences of the NS proteins of a virus of the DENV2, DENV3 or DENV4 serotype or (ii) consists of a chimeric polyepitope having an amino acid sequence which has more than 75 % identity, in particular more than 80% or more than 85% or more than 90% identity with the sequence of the fusion polypeptide consisting of fused fragments (a), (b) and (c) (from which it
25 derives by mutation of amino acid residues), over its whole length.

In a particular embodiment, the present invention relates to a chimeric polyepitope having a size of less than 600 amino acid residues, in particular consisting of a strand of 539 amino acid residues and comprising or consisting of the fusion of fragments named (a), (b) and (c) hereafter and

obtained from or representative of several non-structural (NS) proteins of any DENV genome, namely fragments consisting of:

(a) the NS3 protein consisting of amino acids 1645 to 1829 and 1959 to 2092 in the polyprotein sequence of DENV1 (Genbank accession number NP_059433.1), or amino acids 1645 to 1828 and 1958 to 2091 in the polyprotein sequence of DENV2 (Genbank accession number NP_056776.2), or amino acids 1643 to 1827 and 1957 to 2090 in the polyprotein sequence of DENV3 (Genbank accession number YP_001621843.1), or amino acids 1644 to 1827 and 1957 to 2090 in the polyprotein sequence of DENV4 (Genbank accession number NP_073286.1),

(b) the NS4b protein consisting of amino acids 2262 to 2347 in the polyprotein sequence of DENV1 (Genbank accession number NP_059433.1), or amino acids 2260 to 2345 in the polyprotein sequence of DENV2 (Genbank accession number NP_056776.2), or amino acids 2260 to 2344 in the polyprotein sequence of DENV3 (Genbank accession number YP_001621843.1), or amino acids 2259 to 2341 in the polyprotein sequence of DENV4 (Genbank accession number NP_073286.1), and

(c) the NS5 protein consisting of amino acids 2766 to 2899 in the polyprotein sequence of DENV1 (Genbank accession number NP_059433.1), or amino acids 2765 to 2898 in the polyprotein sequence of DENV2 (Genbank accession number NP_056776.2), or amino acids 2763 to 2896 in the polyprotein sequence of DENV3 (Genbank accession number YP_001621843.1), or amino acids 2761 to 2894 in the polyprotein sequence of DENV4 (Genbank accession number NP_073286.1),

wherein each fragment (a), (b) and (c) comprises a plurality of epitopes suitable for the elicitation of an immune response, especially an immune T-cell response against all DENV serotypes, said polyepitope resulting from direct or indirect fusion of the plurality of said fragments, in particular said fragments originating from a unique dengue virus serotype.

In a particular embodiment, the present invention relates to a chimeric polypeptide having a size of 539 amino acids, and comprising or consisting of the fusion of fragments named (a), (b) and (c) hereafter and obtained from or representative of several non-structural (NS) proteins of any DENV genome,
5 namely fragments consisting of:

(a) the NS3 protein encoded by nucleotides 5027 to 5581 and 5969 to 6370 in the nucleotide sequence of DENV1 (Genbank accession number NC_001477.1), or nucleotides 5026 to 5580 and 5968 to 6369 in the nucleotide sequence of DENV2 (Genbank accession number NC_001474.2),
10 or nucleotides 5021 to 5575 and 5963 to 6364 in the nucleotide sequence of DENV 3 (Genbank accession number NC_001475.2), or nucleotides 5028 to 5582 and 5970 to 6371 in the nucleotide sequence of DENV 4 (Genbank accession number NC_002640.1),

(b) the NS4b protein encoded by nucleotides 6878 to 7135 in the nucleotide
15 sequence of DENV1 (Genbank accession number NC_001477.1), or nucleotides 6874 to 7131 in the nucleotide sequence of DENV2 (Genbank accession number NC_001474.2), or nucleotides 6872 to 7126 in the nucleotide sequence of DENV 3 (Genbank accession number NC_001475.2), or nucleotides 6876 to 7124 in the nucleotide sequence of
20 DENV 4 (Genbank accession number NC_002640.1), and

(c) the NS5 protein encoded by nucleotides 8390 to 8791 in the nucleotide sequence of DENV1 (Genbank accession number NC_001477.1), or nucleotides 8389 to 8790 in the nucleotide sequence of DENV2 (Genbank accession number NC_001474.2), or nucleotides 8381 to 8782 in the
25 nucleotide sequence of DENV 3 (Genbank accession number NC_001475.2), or nucleotides 8382 to 8783 in the nucleotide sequence of DENV 4 (Genbank accession number NC_002640.1),

wherein each translated fragment (a), (b) and (c) comprises a plurality of epitopes suitable for the elicitation of an immune response, especially an
30 immune T-cell response against all DENV serotypes, said polypeptide

resulting from direct or indirect fusion of the plurality of said fragments in particular said fragments originating from a unique dengue virus serotype.

As defined herein, the term “*polyepitope*” refers to a polypeptide with advantageously at least 3 and in particular at least 5 and preferably more than 10 or more than 13 epitopes identified in DENV1 NS3, NS4b and NS5 proteins, in particular T-cell epitopes of DENV1 NS3, NS4b or NS5 consensus sequence provided in **Figure 1**. Epitopes within the present invention are, either linear or conformational, preferably linear, and are any peptide or polypeptide involved in the induction of a cell-mediated immune response, especially a T cell immune response against a DENV and in particular against anyone of DENV1, DENV2, DENV3, DENV4 or against multiple, in particular all DENV serotypes. Accordingly, epitopes described herein include those which are processed by APC (Antigen Presenting Cells) in a host, especially T epitopes recognized in association with class I MHC (Major Histocompatibility Complex) molecules, such as epitopes which target cells are CD8+ T lymphocytes or T epitopes recognized in association with class II MHC molecules, such as those which target cells are CD4+ T lymphocytes.

The term “*chimeric polyepitope*” means any polyepitopic polypeptide comprising sub-portions of different DENV NS proteins selected among NS3, NS4b and NS5 proteins, for example a polyepitope deriving from a first DENV NS protein and a polyepitope deriving from a second DENV NS protein as defined herein. A polyepitope is also considered to be a chimeric polyepitope if it includes sub-portions deriving from different polyepitopes from the same DENV NS protein, or even from the same polyepitopes from different DENV NS proteins. The chimeric polyepitope of the invention includes the polyepitope variant. Accordingly each definition or embodiment disclosed herein applies to the variant polyepitope unless it is technically irrelevant.

In a particular embodiment of the invention, the chimeric polyepitope comprises human leukocyte antigen (HLA)-restricted epitopes. The

expression “HLA-restricted” refers to the capacity for a particular epitope to have an affinity for this type of HLA molecule. The HLA molecules used in the invention encompass either class I molecules (designated HLA-A, B or C) or class II molecules (designated DP, DQ or DR).

5 In another particular embodiment of the invention, the chimeric polyepitope elicits a human leukocyte antigen (HLA)-restricted CD8⁺ and/or CD4⁺ T cell response against DENV1, DENV2, DENV3 and DENV4. A non-exhaustive list of DENV CD8 T cell epitopes is provided in **Table 1**.

10 **Table 1. DENV CD8 T+ cell epitopes having amino acid sequences as defined in SEQ ID NOs: 16-68.** ^aAmino acids differing from the described epitope are underlined; ^bCD8 T cell epitopes with positive score in the Elispot test are highlighted; ^cNT: not tested. References for HLA restriction are as follows: (1) Rivino L et al., *J Virol* 2013, 87(5):2693-2706; (2) Simmons CP et al. *J Virol* 2005, 79(9):5665-5675; (3) Imrie A et al. *J Virol* 2007, 81(18):10081-10091; (4) Boucherma R et al., *J Immunol* 2013, 191(2):583-593; (5) Weiskopf D et al., *Proc Natl Acad Sci U S A* 2013, 110(22):E2046-2053; (6) Lund O et al., *PLoS ONE* 2011, 6(10):e26494; (7) Rivino L et al., *J Immunol* 2013, 191(8):4010-4019; (8) Nascimento EJ et al., *PLoS neglected*
15 *tropical diseases* 2013, 7(10):e2497; (9) Immune Epitope Database (IEDB);
20 (10) Weiskopf D et al., *J Immunol* 2011, 187(8):4268-4279; (11) Yauch LE et al., *J Immunol* 2009, 182(8):4865-4873.

Nb	HLA restriction (references)	Peptide synthesized ^{a, b}	Position	+ SFC ^c
P20	B35 (5)	<u>L</u> PEIEDE <u>V</u> F	NS3-1 (7-15)	-
P19	B35 (5, 9)	HPGSGKTRRY	NS3-1 (26-35)	-
P30	A31 (1, 7), B7 (6, 9)	RYLPAIVREAI	NS3-1 (34-44)	B7
P451	A2 (10)	YLPAIVREA	NS3-1 (35-43)	A2

P11	A31 (1, 7), B7 (6, 9)	LPAIVREAI	NS3-1 (36-44)	-
P36	B7 (1, 2, 9), B35 (9)	APTRVVASEM <u> </u>	NS3-1 (54-63)	B7
P48	B7 (1, 9), A2,11; B35,62 (3)	EMAEALKGMPIRYQT	NS3-1 (62-76)	-
P12	ND (2), B7 (9)	SPVRVPNYNM	NS3-1 (103-112)	-
P454	B7 (10)	VPNYNMIIM	NS3-1 (107-115)	-
P18	ND (2), B35 (5, 9)	DPSSIAARGY	NS3-1 (122-131)	-
P457	B7 (10)	AVIQDEERDI <u> </u>	NS3-1 (162-171)	-
P453	B58 (1, 7)	RSWNSGYEW <u> </u>	NS3-1 (174-182)	B35
P49	B35 (1, 2, 3, 4, 9)	TPEGIIPAL <u> </u>	NS3-2 (203-211)	B35
P28	A11 (9)	KTFVELMRR <u> </u>	NS3-2 (234-242)	NT
P2	B55 (1), A2 (9)	ELMRRGDLPV	NS3-2 (238-247)	-
P450	A2 (10)	DLMRRGDLPV	NS3-2 (238-247)	-
P50	nd (2), B35 (5)	LPVWLSYKVA <u> </u>	NS3-2 (245-254)	B35
P32	A24 (1, 2, 6, 9, 11)	QYSDRRWCF	NS3-2 (259-267)	A24
P73	A24 (1, 2, 6, 11)	NYADRRWCF	NS3-2 (259-267)	NT
P74	A24 (13)	NYADRKWCF	NS3-2 (259-267)	NT
P75	A24 (13)	KYTDRKWCF	NS3-2 (259-267)	NT
P76	A24 (13)	SYKDREWCF	NS3-2 (259-267)	NT
P35	B7 (8, 9)	RPRWLDART	NS3-2	-

			(295-303)	
P17	A24 (1, 7, 8)	LDARTYSDPLALREFKEF	NS3-2 (299-316)	A24
P29	A24 (1, 7, 8)	RIYSDPLALK	NS3-2 (302-311)	-
P452	A11, A24 (1, 7, 8)	RTYSDPLALR	NS3-2 (302-311)	NT
P21	B7, B35 (9)	HPASAWTLY	NS4b (336-344)	B35
P16	B7, B35 (9)	PASAWTLY	NS4b (337-344)	-
P13	B35 (9)	HPASAWTLYA	NS4b (336-345)	NT
P51	A2 (6) B35 (9)	TLYAVATTI	NS4b (342-350)	B35
P52	B35 (9)	VATTIITPM	NS4b (346-354)	-
P33	A24 (9)	ITPMMRHTI	NS4b (351-359)	A24
P14	A24 (9)	TPMMRHTIEN	NS4b (352-361)	-
P22	B35 (5, 9)	IANQAAILM	NS4b (371-379)	-
P456	A11 (10)	AAILMGLDK	NS4b (375-383)	NT
P53	A2 (6)	KMDIGVPLL	NS4b (389-397)	-
P54	B35 (9)	VPLLALGCV	NS4b (394-402)	-
P55	A2,11, B35,62 (3)	DNPYKTWAYH	NS5 (429-438)	-
P24	B35 (9)	WAYHGSEV	NS5 (435-443)	-
P56	A2 (6)	SMVNGVVKL	NS5 (435-443)	A2
P5	B55 (1), A2,24,	LLTKPWDVIPMVTQI	NS5	-

	B55,61 (3), A2 (8)		(459-473)	
P6	“	LLTKPWDVIP	NS5 (459-468)	-
P7	“	LTKPWDVIPM	NS5 (460-469)	-
P8	“	TKPWDVIPMV	NS5 (461-470)	-
P9	“	KPWDVIPMVT	NS5 (462-471)	-
P10	“	PWDVIPMVTQ	NS5 (463-472)	-
P27	“	WDVIPMVTQI	NS5 (464-473)	-
P455	B7 (9)	<u>IPMVTQIAM</u>	NS5 (467-475)	-
P34	A68, B35 (1)	DTPFGQQR	NS5 (477-485)	-
P23	B35 (9)	TPFGQQRVF	NS5 (479-487)	-
P3	A2,32, B35,62 (3), B7 (7, 8)	TAKWLWGFLSRNKKPRICTR	NS5 (509-528)	-
P4	A2,32, B35,62 (3)	WGFLSRNKK	NS5 (514-522)	-
P15	B7 (5, 8, 9)	KPRICTREEF	NS5 (522-531)	B7

In another particular embodiment of the invention, the chimeric polypeptide elicits a human leukocyte antigen (HLA)-restricted CD4⁺ T cell response against DENV1, DENV2, DENV3 and DENV4. A non-exhaustive
5 list of DENV CD4 T cell epitopes is provided in **Table 2**.

Table 2. DENV CD4 T+ cell epitopes having amino acid sequences as defined in SEQ ID NOs: 69-97.

HLA Class II restriction (references)	Peptide sequence	Position
HLA-DR2 (Nascimento, E., 2013)	PELEEEMFKKRNLTI	NS3-1 (8-22)
Maloy M. Mangada, 2005	RKLTIMDLHPGSGKT	NS3-1 (18-32)
HLA-DR15 (Simmons, et al., 2006)	TKRYLPAIVREAIKR	NS3-1 (32-46)
HLA-DR15 (Zeng, L., et al., 1996)	RKYLPAIVRE	NS3-1 (33-42)
HLA-DR3 (Nascimento, E., 2013)	LP AIVREAIKRRLRT	NS3-1 (36-50)
HLA-DR3 (Nascimento, E., 2013)	VREAIKRRLRTLILA	NS3-1 (40-54)
HLA-DR15 (Kurane, I., 1998)	TRVVAAEMEEA	NS3-1 (56-66)
HLA-DRB1*15:01 (Maloy M. Mangada, 2005)	PTRVVAAEMEEAMKG	NS3-1 (55-69)
HLA-DR15 (Moran, E., 2008)	ALKGMPYRYQTTAVK	NS3-1 (66-80)
HLA-DR4 (Nascimento, E., 2013)	KGLPIRYQTTATKSE	NS3-1 (68-82)
HLA-DR4 (Nascimento, E., 2013)	IRYQTTATKSEHTGR	NS3-1 (72-86)
HLA-DPw2 (Maloy M. Mangada, 2005)	HTGREIVDLMCHATE	NS3-1 (83-97)
HLA-DPA1*01:03/DPB1*02:01 (Falta, MT., 2013)	REIVDLMCHATF	NS3-1 (86-97)
HLA-DPw2 (Kurane, I., et al., 1993)	EIVDLMCHAT	NS3-1 (87-96)
HLA-DPw2 (Okamoto, Y., 1998)	VDLMCHATFT	NS3-1 (89-98)
HLA-DRB1*01:01 (Weiskopf, D., 2013)	TFTMRLLSPVRVPNY	NS3-1 (96-110)
Rivino, L., 2013	PNYNLIIMDEAHFTD	NS3-1 (108-122)
HLA-DR2 (Nascimento, E., 2013)	ASIAARGYISTRVGM	NS3-1 (124-138)
HLA-DR4 (Nascimento, E., 2013)	EAAAI FMTATPPGTA	NS3-1 (139-154)
Maloy M. Mangada, 2005	REGKKKKLRPRWLDR	NS3-2 (287-301)
HLA-DR2 (Nascimento, E., 2013)	PLALKEFKDFAAGRK	NS3-2 (307-319)
HLA-DR3 (Nascimento, E., 2013)	KEEHSSTWHYDDENPYK	NS5 (417-433)
HLA-DR2 (Nascimento, E., 2013)	TWHYDDENPYKTWAYHG	NS5 (423-439)
HLA-DR2 (Nascimento, E., 2013)	DENPYKTWAYHGSYEVK	NS5 (427-444)
Rivino et al., 2013	KTWAYHGSYETKQTG	NS5 (433-447)
HLA-DR2 (Nascimento, E., 2013)	SMINGVVKLLTKPWDVV	NS5 (451-473)
Imrie, A., 2007	KPWDVLP MV	NS5 (462-471)
HLA-DR4 (Nascimento, E., 2013)	VKLLTKPWDVVPMVTQM	NS5 (457-463)

HLA-DR4 (Nascimento, E., 2013)	MVTQMAMTDTTPFGQQRv	NS5 (469-485)
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In a particular embodiment, the NS chimeric polyepitope sequence has been shown to elicit antigenic responses in mice with HLA restriction such as HLA-A*02:01, HLA-A*24:02, HLA-B*07:02 or HLA-B*35:01.

5 In a particular embodiment, the present invention relates to a chimeric polyepitope, whose size is 539 amino acids, comprising or consisting of:

(a) two fragments of the non-structural (NS) NS3 protein of the dengue virus (DENV) serotype 1 (DENV1), having amino acid sequences as defined in SEQ ID NO: 6, SEQ ID NO: 9, respectively,

10 (b) a fragment of the NS4b protein of DENV1 comprising amino acid sequences as defined in SEQ ID NO: 12,

(c) a fragment of the NS5 protein of DENV1 comprising amino acid sequences as defined in SEQ ID NO: 15, and

15 wherein each NS fragment (a), (b) and (c) is fused directly or indirectly with another NS fragment (a), (b) and (c) in the polyepitope, preferably in this order.

Nucleotide sequences of the polyprotein of DENV1, DENV2, DENV3 and DENV4 can be accessed from the Genbank accession numbers NP_059433.1, NP_056776.2, YP_001621843.1 and NP_073286.1
20 respectively.

Amino acid sequences of DENV2-NS, DENV3-NS and DENV4-NS polyepitopes of SEQ ID NO: 146, 147 and 148 respectively which are variant polyepitopes of the invention are disclosed in **Figure 3**. The inventors have carried out an alignment showing conserved amino acid residues in
25 sequences of DENV1-NS, DENV2-NS, DENV3-NS and DENV4-NS polyepitopes (**Figure 4**).

In a preferred embodiment of the invention, the chimeric polyepitope comprises at least the P30, P451, P36, P453, P49, P50, P32, P17, P21, P51, P33, P56 and P15 epitopes disclosed herein. In particular, the P30, P451
30 and P56 epitopes are restricted by HLA-A*02:01, the P17, P32 and P33

epitopes are restricted by HLA-A*24:02, the P15, P30 and P36 epitopes are restricted by HLA-B*07:02 and the P21, P49, P50, P51 and P453 epitopes are restricted by HLA-B*35:01. The chimeric polyepitope is expected to contain a number of additional epitopes at least equal to the number of existing HLA, with examples provided in **Tables 1 and 2**.

The epitopes of the invention can have amino acid sequences that are distinct or that differ by one or more amino acids in the consensus NS determined for DENV1 within the frame of the invention. Alternatively or in addition, two epitopes of the polyepitope of the invention can have overlapping sequences in one NS fragment, and accordingly share some amino acids.

Chimeric polyepitopes of the invention can be synthesized chemically, or produced either *in vitro* (cell free system) or *in vivo* after expression of the nucleic acid molecule encoding the polyepitope in a cell system.

As defined herein, the term “*fragment*” refers to parts or portions of NS proteins (*i.e.* NS3, NS4b or NS5 protein), in particular portions having from 86 to 185 amino acids. Any sequence or combination of sequences of any dengue virus isolate corresponding to the fragments of the invention (as delimited by the positions in the NS proteins as identified using nucleotide and amino acid numbering on DENV1 reference sequence deposited in Genbank (NP_059433.1) or as disclosed with respect to its SEQ ID NO. is shorter in length than the NS protein from which it originates.

According to a particular embodiment of the invention, one NS fragment is fused “*directly*” with another NS fragment, *i.e.* the 3' end of the NS fragment is directly linked to the 5' end of the second fragment (and so on), corresponding to a chimeric polyepitope composed of consecutive NS fragments from the same and/or from different NS proteins chosen among NS3, NS4b and NS5, in particular originating from NS consensus sequence of DENV1. According to an alternative embodiment, the fusion of the fragments is “*indirect*” and accordingly involves the presence of non-NS amino acid residues segment(s), *i.e.*, amino acid residues segments which

do not read on the NS protein providing the sequence of the considered fragment.

As defined herein, the term “*region*” refers to contiguous amino acid strand of a NS protein as defined herein, having at least 86 amino acids. A
5 fragment of a NS protein may comprise or consist of a plurality of regions as for the NS3 fragment.

The term “*percentage identity*” between two compared nucleotidic sequences or respectively two amino acid sequences as used in the present invention means a percentage of identical nucleotides or amino acids
10 between the two sequences to be compared, obtained after best alignment, that percentage being purely statistical and the differences between the two sequences being randomly distributed and over their entire length. The term “best alignment” or “optimum alignment” means the alignment at which the percentage of identity is the highest. Sequence comparisons between two
15 nucleic acid or amino acid sequences are traditionally carried out by comparing these sequences after having aligned them in an optimum manner, said comparison being carried out using comparison segments or windows to identify and compare local regions with sequence similarity. The optimum sequence alignment for comparison may be carried out manually or
20 using a Smith and Waterman (1981) local homology algorithm, using the Needleman and Wunsch (1970) local homology algorithm, using the Pearson and Lipman (1988) sequence similarity search method, or using software employing these algorithms (GAP, BESTFIT, BLAST P, BLAST N, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics
25 Computer Group, 575 Science Dr, Madison, WI). To obtain an optimal alignment, the BLAST program may be used, with the BLOSUM 62 matrix. It is also possible to use PAM or PAM250 matrices. A total of 2033 full-length dengue genome sequences (865 for serotype 1, 678 for serotype 2, 427 for serotype 3 and 63 for serotype 4) were aligned using MUSCLE 3.7 (*Edgar RC, Nucleic Acids Res* 2004, 32(5):1792-1797), with manual adjustments
30 according to the amino acid sequence.

The percentage identity between two nucleic acid or two amino acid sequences is determined by comparing these two sequences aligned in an optimum manner. The percentage identity is calculated by determining the number of identical positions for which the nucleotide or amino acid residue is identical between the two sequences, dividing this number of identical positions by the total number of compared positions and multiplying the result obtained by 100 to obtain the percentage identity between these two sequences.

In a particular embodiment of the invention, the first region of NS3 of DENV1 has an amino acid sequence as defined in SEQ ID NO: 6, the second region of NS3 of DENV1 has an amino acid sequence as defined in SEQ ID NO: 9, the NS4b fragment of DENV1 has an amino acid sequence as defined in SEQ ID NO: 12 and the NS5 fragment of DENV1 has an amino acid sequence as defined in SEQ ID NO: 15.

In another particular embodiment of the invention, the native and optimised sequences of the polynucleotide encoding the first region of NS3 of DENV1 are as defined in SEQ ID NOs: 4 and 5 respectively, the native and optimised sequences of the polynucleotide encoding the second region of NS3 of DENV1 are as defined in SEQ ID NOs: 7 and 8 respectively, the native and optimised sequences of the polynucleotide encoding the NS4b fragment of DENV1 are as defined in SEQ ID NOs: 10 and 11 respectively, and the native and optimised sequences of the polynucleotide encoding the NS5 fragment of DENV1 are as defined in SEQ ID NOs: 13 and 14 respectively.

The term "*variants*" encompasses the assembly of the other fragments of the NS3, NS4b and NS5 proteins of a virus of the DENV serotype 2, 3 or 4, as disclosed herein.

In a preferred embodiment of the invention, the chimeric polypeptide comprises or consists of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 146, 147 and 148.

The present invention also relates to an association of the chimeric polyepitope of the invention, with a distinct immunogenic polypeptide hereafter designated as tetravalent EDIII/ectoM. In a particular embodiment of the invention, said distinct immunogenic polypeptide (tetravalent
5 EDIII/ectoM) consists of chimeric DENV antigens composed of the fusion of the EDIII polypeptides representative of the four DENV serotypes, fused to ectoM of DENV1, and has an amino acid sequence as disclosed in SEQ ID NO: 145, and is encoded by the polynucleotide having SEQ ID NO: 144.

Said association of polypeptides may be achieved as a result of
10 expression of the polynucleotides encoding each of said chimeric polyepitope and distinct immunogenic polypeptide, from a vector as disclosed herein. Alternatively, said association may result from an amino acid construct encompassing said chimeric polyepitope and distinct immunogenic polypeptide.

15 The present invention also relates to viral particles, in particular recombinant measles virus (MV) particles expressing a chimeric polypeptide of the invention or said chimeric polypeptide in association with the tetravalent EDIII/ectoM polypeptide.

The present invention also relates to a vector comprising or consisting
20 of recombinant measles virus (MV) particles expressing the chimeric polyepitope of the invention and optionally the tetravalent EDIII/ectoM immunogenic polypeptide.

The thus produced recombinant viral particles, in particular measles virus particles have proved to enhance the immune response elicited by the
25 DENV antigens when used alone, in particular by impacting the immunogenic T-cell response elicited following administration of the particles to a host in need thereof.

In order to prepare the viral particles, in particular MV particles expressing the chimeric polyepitope of the invention in association with the
30 so-called tetravalent EDIII/ectoM polypeptide as disclosed herein, the polynucleotide(s) encoding said polyepitope and polypeptide are recombined

into a polynucleotide encompassing the genome of the virus, especially the genome of the measles virus.

According to a preferred embodiment of the invention, the recombinant MV genome is provided as a cDNA encoding the full-length RNA genome of
5 a live-attenuated Schwarz or Moraten virus.

A live-attenuated MV strain refers to a strain which has been serially passaged on selected cells and, preferably, adapted to other cells such as primary cells with an IFN α/β response, *i.e.* CEF cells, to produce seed strains suitable for the preparation of vaccine strains, harboring a stable
10 genome which would not allow reversion to pathogenicity nor integration into host chromosomes, in particular human host chromosomes. In a particular embodiment of the invention, a live-attenuated MV is one which has been selected on primary cells such as CEF cells.

As defined herein, the expression "*live-attenuated Schwarz or Moraten virus*" designates a Schwarz or Moraten virus originating from a strain that is
15 avirulent or less virulent than a determined parent strain in the same host, especially in human, while maintaining infectious properties and immunogenicity and possibly adjuvancy when administered in a host, especially in human. In particular, the Schwarz strain or the Moraten strain is
20 from the Rouvax® vaccine (Aventis). It has been demonstrated that the Schwarz strain has a perfect identity of sequence with the Moraten strain (*Parks, C. L. et al., 2001, J Virol, 75(2): 910-920; Schwarz, A. J., 1962, Am J Dis Child, 103, 386-389*). The Schwarz/Moraten strains are currently widely-used since they induce long-term cell and humoral immune responses and
25 present an important genetic stability since no reversion to a pathogenic form has ever been observed (*Hilleman, M., 2002, Vaccine, 20:651-665*).

The measles virus genome is obtained as a result of preparation of a cDNA molecule encoding the full-length MV genome as disclosed in the art especially in WO 2004/001051 and WO 2004/000876. Said cDNA molecule
30 has been included as an insert in a plasmid designated pTM-MVSchw deposited on June 12, 2002 with the CNCM under No. I-2889 (Institut Pasteur-Paris-France). The sequence of the MV cDNA encoding the genome

is provided in the figures illustrating the constructs prepared with the chimeric polyepitope of the invention and the distinct so-called tetravalent EDIII/ectoM polypeptide. The position of the MV sequences is disclosed in said figures.

In order to express the chimeric polyepitope of the invention and
5 optionally the distinct so-called tetravalent EDIII/ectoM polypeptide, said cDNA molecule encoding the full-length MV genome is recombined with a DNA molecule encoding the chimeric polyepitope of the invention and optionally in a DNA molecule encoding the so-called tetravalent EDIII/ectoM polypeptide as disclosed herein, using methods and protocols well-known
10 from the person skilled in the art. The DNA molecules encoding said polypeptides are prepared according to known techniques, by the skilled person.

In a particular embodiment, a DNA molecule corresponding to the chimeric polyepitope of the invention can be generated by chemical
15 synthesis. Preferably, this sequence is codon-optimized for expression in mammalian cells and preferably its length fulfills the “rule of six” which stipulates that the total number of nucleotides is a multiple of 6, which rule is known to impact the proper expression of the MV proteins from the MV genome (*Calain, P. et al. J Virol.*, 1993, 67:4822-4830).

20 In a particular embodiment, all nucleic acid constructs inserted in the cDNA encoding the full-length MV genome, in particular coding sequences for the chimeric polyepitope and for the so-called tetravalent EDIII/ectoM polypeptide have a number of nucleotides which is a multiple of six.

The present invention thus relates to an isolated or purified
25 polynucleotide encoding the chimeric polyepitope of the invention, and having preferably a total number of nucleotides which complies with the rule of six. This polynucleotide is in particular a DNA or a cDNA. In a particular embodiment, MV editing- and polyadenylation-like sequences of the sequence encoding the NS fragments of the polynucleotide of the invention
30 are mutated (Lamb, R. A. et al. In *Fields Virology*, 4th edition, 1305–1340; *Schneider, H. et al. Virology*, 1997, 227: 314-322). In addition, to allow its future cloning into a plasmid such as pUC into a mammalian expression

plasmid such as pcDNA3, or into MV vector, the DNA encoding the chimeric polyepitope of the invention was flanked by sequences of restriction sites such as BglII, ApaI, BsiWI and BssHII.

The invention accordingly concerns in particular the native and codon
5 optimized nucleotide sequences encoding the chimeric polyepitope of DENV1 which are the sequences disclosed as SEQ ID NO: 1 and SEQ ID NO: 2 respectively (**Figures 5 and 6**). Amino acid sequence of the chimeric polyepitope of DENV1 is the sequence disclosed as SEQ ID NO: 3.

The present invention also relates to an isolated or purified
10 polynucleotide encoding the chimeric polyepitope of the invention, in a nucleic acid construct further comprising a polynucleotide encoding tetravalent DENV antigens composed of the fusion of the EDIII polypeptides of the four serotypes, fused to ectoM of DENV1, wherein the polynucleotide obtained has preferably a total number of nucleotides which complies with
15 the rule of six. In a particular embodiment, this polynucleotide has the sequence of SEQ ID NO: 144. Such a construct may in particular comprise the polynucleotide encoding tetravalent DENV antigens composed of the fusion of the EDIII polypeptides of the four serotypes, fused to ectoM of DENV1 upstream from the polynucleotide encoding the chimeric polyepitope
20 of the invention.

The invention also relates to a nucleic acid molecule comprising the herein disclosed polynucleotide encoding the chimeric polyepitope of the invention and optionally comprising the polynucleotide encoding tetravalent DENV antigens composed of the fusion of the EDIII polypeptides of the four
25 serotypes, fused to ectoM of DENV1 recombined with the cDNA molecule encoding the full-length MV genome.

As defined herein, the term "*isolated or purified*" means molecules which have been altered by man from their native state, *i.e.* if the molecules exist in nature, they have been changed and/or withdrawn from their initial
30 environment. As an example, a polynucleotide naturally present and found in the biological environment of a living organism which naturally expresses it is not "isolated" in this context. However, the same polynucleotide when

separated from its natural environment and/or obtained by cloning, amplification and/or chemical synthesis is considered in the present invention to be "isolated". Further, a polynucleotide which is introduced into an organism by transformation, gene manipulation or any other recombination method is "isolated" even if it is present in said organism.

The term "*encoding*" used in the present application defines the ability of the nucleic acid molecules to be transcribed and where appropriate translated for product expression into selected cells or cell lines, when said molecule is placed under expression control sequences including promoter for transcription. Accordingly a "*polynucleotide encoding*" according to the invention is either limited to the nucleic acid having the sequence translated into the amino acid sequence or alternatively when specified comprises also the expression control sequences.

The invention also relates to a vector. As used herein, the term "*vector*" refers to a polynucleotide construct designed for transduction/transfection of one or more cell types. Vectors may be, for example, "cloning vectors" which are designed for isolation, propagation and replication of inserted polynucleotides (designated as the insert), "expression vectors" which are designed for expression of a polynucleotide molecule especially for expression of the insert in a host cell, or a "viral vector" which is designed to result in the production of recombinant virus particles or virus-like particles, or "shuttle vectors", which comprise the attributes of more than one type of vector.

A number of vectors suitable for transduction or for transfection of cells, in particular for stable transfection of cells and bacteria are available to the public (e.g. plasmids, viruses), as are methods for constructing such cell lines. It will be understood that the present invention encompasses any type of vector comprising any of the polynucleotides of the invention.

The present invention accordingly relates to a vector, in particular an expression vector, which may be a plasmid comprising as polynucleotide insert(s), one or a plurality of the nucleic acid molecules defined herein. In a particular embodiment, the plasmid comprises as an insert a polynucleotide

encoding the chimeric polyepitope of the invention as defined herein and optionally comprises the polynucleotide encoding tetravalent DENV antigens composed of the fusion of the EDIII polypeptides of the four serotypes, fused to ectoM of DENV1.

5 In a particular embodiment, the present invention concerns a plasmid (designated measles genome vector) comprising (i) a cDNA encoding the full-length RNA genome of a measles virus (MV), which cDNA is recombined with (ii) a polynucleotide according to the invention encoding the chimeric polyepitope of the invention as defined herein and optionally (iii) a
10 polynucleotide encoding tetravalent DENV antigens composed of the fusion of the EDIII polypeptides of the four serotypes, fused to ectoM of DENV1 as defined herein. In a particular embodiment when the sequences of (i), (ii) and optionally (iii) are present in the MV genome vector, they comply together with the rule of six.

15 In a particular embodiment said polynucleotide(s) is(are) located as separate insert(s) between the P and M genes of the MV genome, or between the H and L genes of the MV genome. Optionally said insert(s) is(are) located in an Additional Transcription Unit (ATU) such as the ATU having the sequence of ATU 2 or ATU3 illustrated in the construct MDVVax6.
20 The location of the ATU within the cDNA derived from the antigenomic RNA of MV can vary along said cDNA.

 In a particular embodiment, the present invention concerns a recombinant measles virus (MV) genome vector, which is a plasmid comprising (i) a cDNA encoding the full-length RNA genome of a MV virus
25 and (ii) the polynucleotide encoding the chimeric polyepitope according to the invention, said polynucleotide being located as an insert between the P and M genes of the MV genome or between the H and L genes of the MV genome, optionally in an Additional Transcription Unit (ATU), wherein the sequences of (i) and (ii) when recombined in the plasmid together comply
30 with the rule of six .

The present invention also concerns a recombinant MV genome vector, which is a plasmid comprising (a) a cDNA encoding the full-length RNA genome of a MV virus, (b) a polynucleotide encoding tetravalent DENV antigens composed of the fusion of the envelope domain III (EDIII) polypeptides of the four DENV serotypes, fused to the ectodomain of the membrane protein (ectoM) of DENV1, in particular DENV antigens having the sequence of SEQ ID NO: 145 and (c) the polynucleotide according to the invention, wherein:

- the polynucleotide (b) is located as an insert between the P and M genes of the MV genome and the polynucleotide (c) is located as an insert between the H and L genes of the MV genome, or

- the polynucleotide (b) is located as an insert between the P and M genes of the MV genome and the polynucleotide (c) is located as an insert between the P and M genes of the MV genome, or

- the polynucleotide (b) is located as an insert between the H and L genes of the MV genome and the polynucleotide (c) is located as an insert between the P and M genes of the MV genome and

wherein the sequences of (a), (b) and (c) when recombined in the plasmid together comply with the rule of six.

In a particular embodiment, when the sequences of (a), (b) and (c) are recombined in a plasmid they together comply with the rules of six.

The nucleotide sequences of particular vectors of the invention comprising both polynucleotides are the sequences disclosed as SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143 and SEQ ID NO: 149.

The present invention also relates to a host cell genetically transformed with the polynucleotide encoding the chimeric polyepitope of the invention and optionally with the polynucleotide encoding the tetravalent EDIII/ectoM according to the invention, in particular a polynucleotide encoding tetravalent DENV antigens composed of the fusion of the envelope domain III (EDIII) polypeptides of the four DENV serotypes, fused to the

ectodomain of the membrane protein (ectoM) of DENV1. A particular host cell may thus be genetically transformed with a vector of the invention, in particular with a recombinant MV genome vector of the invention.

5 The host cell of the invention is transfected with a genome vector of the invention, by methods well known to the man skilled in the art, *i.e.* by chemical transfection (calcium phosphate, lipofectamine), lipid-based techniques (liposome), electroporation, photoporation, use of viral vectors....

10 In a particular embodiment, a cell is transformed or transduced with a polynucleotide of the invention, in a way enabling integration of the polynucleotide in the cell genome either by a recombination with the homologous cellular sequence or by insertion in the cellular genome. The transfection, infection or transduction can occur *ex vivo*, *i.e.* in an artificial environment outside the living organism.

15 As used herein, the terms “*transfected*”, “*transformed*” or “*infected*” refer to a cell comprising a vector of the invention (transient expression), whereas the term “*genetically transformed*” refers to a cell whose genome has been definitively modified by a polynucleotide of the invention (permanent expression).

20 Said transitory or stably transformed cells can be any prokaryotic (bacteria) or eukaryotic (yeast, insect or animal including mammal especially human) cells. In an embodiment, cells are non-human cells. In a particular embodiment, cells of the invention are isolated human cells, “*isolated*” meaning outside of their natural environment.

25 In a particular embodiment of the invention, the cell is HEK-293-T7-MV cell line.

More preferably, an infectious recombinant MV genome vector suitable to carry out the invention is produced using a cDNA of MV Schwarz strain cloned into the plasmid pTM-MV Schw, deposited by Institut Pasteur at the CNCM (Paris, France) under number I-2889 on June 12, 2002, the sequence
30 of which is described by Combredet (*Combredet, C. et al., 2003, J Virol*,

77(21): 11546-11554), and also disclosed in WO2004/000876. The plasmid pTM-MV Schw has been obtained from a Bluescript plasmid, and comprises the polynucleotide coding for the full-length MV (+) RNA strand of the Schwarz strain placed under the control of the promoter of the T7 RNA polymerase, and has 18967 nucleotides. cDNAs from other MV strains may be similarly obtained starting from the nucleic acid purified from viral particles of live-attenuated MV. Accordingly a recombinant MV genome vector of the invention is pTM-MVDVax6 as illustrated by SEQ ID NO: 139, pTM-MVDVax7 as illustrated by SEQ ID NO: 140, pTM-MVDVax8 as illustrated by SEQ ID NO: 141, pTM-MVDVax9 as illustrated by SEQ ID NO: 142, pTM-MVDVax10 as illustrated by SEQ ID NO: 143 or pTM-MVDVax11 as illustrated by SEQ ID NO: 149.

The plasmid comprising the recombinant MV genome thus defined (genome vectors) are suitable for use in a rescue system for the preparation of recombinant measles virus particles.

Rescue of recombinant MV viruses can be performed as previously described (*Combredet et al., J Virol., 2003, 77(21): 11546-11554*), in particular using stable HEK293-T7-MV helper cells (WO2004/000876).

In order for the rescue of recombinant MV particles to be achieved and enable the assembly of recombinant MV particles of the invention, helper cells (host cells) are used which express an RNA polymerase such as the T7 RNA polymerase and the N, P and L proteins of MV and which are genetically transformed with a MV genome vector according to the invention. The expression of the MV proteins by the helper cell may be obtained by genetically transforming the helper cell with additional, vectors such as transcomplementation plasmids, expressing an RNA polymerase such as the T7 RNA polymerase and the N, P and L proteins of MV.

In a particular embodiment of the invention, the helper cell line is the 293-T7-NP cell line deposited with the Collection Nationale de Cultures de Micro-organismes (CNCM) on June 14, 2006, under number I-3618 or the 293-nlsT7-NP MV cell line deposited with the CNCM on August 04, 2006, under number I-3662.

The present invention also relates to recombinant MV particles, which are rescued from a helper cell line expressing the T7 RNA polymerase and the N, P and L proteins of MV, and which further expresses a nucleic acid encoding a vector of the invention or a nucleic acid encoding a recombinant
5 MV genome vector of the invention.

In a further aspect, the present invention relates to an immunogenic composition comprising (a) DENV antigens composed of the fusion of the EDIII polypeptides of the four DENV serotypes, fused to ectoM of DENV1, in particular DENV antigens having the sequence of SEQ ID NO: 145 and (b)
10 the chimeric polyepitope according to the invention, which optionally does not comprise an accessory adjuvant.

The present invention also relates to an immunogenic composition comprising recombinant MV particles according to the invention, which composition optionally does not comprise an accessory adjuvant.
15 Interestingly, the recombinant MV particles of the invention are capable of eliciting a humoral and/or a cellular immune response(s) against the dengue virus infection or against both MV and the dengue virus infection, in a host upon administration. In a particular embodiment, the immunogenic composition of the invention elicits an immune response, in particular a T-cell
20 immune response against the DENV1, DENV2, DENV3 and DENV4 and accordingly may elicit a protective response against infection by any virus of these DENV 1 to 4 serotypes.

According to a particular embodiment of the invention, the immunogenic composition is formulated for an administration through
25 parenteral route such as subcutaneous (s.c.), intradermal (i.d.), intramuscular (i.m.), intraperitoneal (i.p.) or intravenous (i.v.) injection.

According to another particular embodiment of the invention, the immunogenic composition is administered in one or multiple administration dose(s), in particular in a prime-boost administration regime.

30 In a particular embodiment, the immunogenic composition does not comprise an accessory adjuvant.

The quantity to be administered (dosage) depends on the subject to be treated, including the condition of the patient, the state of the individual's immune system, the route of administration and the size of the host. Suitable dosages range from 10^3 TCID₅₀ to 10^7 TCID₅₀ and can be modified by one skilled in the art, depending on circumstances.

The present invention also relates to a vaccine composition comprising (a) DENV antigens composed of the fusion of the EDIII polypeptides of the four DENV serotypes, fused to ectoM of DENV1, and (b) the chimeric polypeptide according to the invention, which optionally does not comprise an accessory adjuvant.

The present invention also relates to a vaccine composition comprising recombinant MV particles according to the invention, which optionally does not comprise an accessory adjuvant.

The present invention also relates to a method to prevent and/or treat a dengue virus infection in a human subject comprising administering a pharmaceutically effective quantity of recombinant MV particles according to the invention or an immunogenic composition according to the invention, wherein said particles or composition are in admixture with a pharmaceutically acceptable vehicle; and/or an adjuvant.

As used herein, the term "*to prevent*" refers to a method by which a dengue virus infection is obstructed or delayed.

As used herein, the term "*to treat*" refers to a method by which the symptoms of a dengue virus infection are either alleviated, *i.e.* decrease of the dengue virus infection in the host or improvement of the clinical condition of the patient, or completely eliminated.

As defined herein, a pharmaceutically acceptable vehicle encompasses any substance that enables the formulation of the chimeric polypeptide, the polynucleotide, the vector, in particular the recombinant MV genome vector according to the invention within a composition. A vehicle is any substance or combination of substances physiologically acceptable *i.e.*, appropriate for its use in a composition in contact with a host, especially a human, and thus non-toxic. Examples of such vehicles are phosphate

buffered saline solutions, distilled water, emulsions such as oil/water emulsions, various types of wetting agents sterile solutions and the like.

As defined herein, an adjuvant includes, for example, liposomes, oily phases, such as Freund type adjuvants, generally used in the form of an emulsion with an aqueous phase or can comprise water-insoluble inorganic salts, such as aluminium hydroxide, zinc sulphate, colloidal iron hydroxide, calcium phosphate or calcium chloride.

The present invention also relates to a method to produce recombinant MV particles for the preparation of an anti-dengue virus vaccine, comprising or consisting of:

- a) transfecting a recombinant MV genome vector according to the invention, in a helper cell line which expresses the T7 RNA polymerase and the N, P and L proteins of MV;
- b) transferring said helper cell line onto cells competent to sustain the replication and production of recombinant MV particles expressing a chimeric polypeptide according to the invention and optionally a polypeptide comprising a tetravalent DENV antigen composed of the fusion of the EDIII polypeptides of the four serotypes, fused to ectoM of DENV1, in particular DENV antigens having the sequence of SEQ ID NO: 145; and
- c) recovering the recombinant MV particles produced in step b).

In a preferred embodiment of the invention, the competent cells in step b) are Vero (African green monkey kidney) cells, CEF (chick embryo fibroblast) cells or MRC5 cells.

EXAMPLES

Cell culture

Vero cells were maintained in DMEM Glutamax (Gibco-BRL) supplemented with 5% heat-inactivated fetal calf serum (FCS, Invitrogen, Frederick, MD). Stable HEK293-T7-MV helper cells used for viral rescue (WO2004/000876) were grown in DMEM supplemented with 10% FCS.

Construction and rescue of recombinant MV vectors

The plasmid pTM-MV Schw, which contains an infectious MV cDNA corresponding to the anti-genome of the Schwarz MV vaccine strain, has been previously described (*Combredet et al., J Virol., 2003, 77(21): 11546-11554*). The synthetic cDNA encoding for the polyepitopic construct of non-structural proteins from DENV1 was inserted into BsiWI/BssHII-digested pTM-MV Schw vectors in different positions, in combination or not with tetrameric EDIII antigen previously described (*Brandler et al., PLoS 2007, 1(3), e96; Brandler et al., Vaccine, 2010, 28, 6730-6739*) (**Figure 7**). Rescue of recombinant viruses was performed as previously described (*Combredet et al., J Virol., 2003, 77(21): 11546-11554*) using stable HEK293-T7-MV helper cells (*WO2004/000876*). The recombinant viruses were grown on Vero cells and viral titers were determined by end point dilution assay on Vero cells.

Western blot

To evidence the expression of DENV antigens by MV vectors or pcDNA3 expression plasmid, lysates from Vero cells infected with MV-DENV or from HEK293 cells transfected with pcDNA3-NS plasmid were fractionated by SDS-PAGE, transferred to cellulose membranes (Amersham Pharmacia Biotech), and probed with anti-EDIII DENV1 mAb 4E11 or anti-NS3 antibody. A goat anti-mouse IgG-horseradish peroxidase (HRP) conjugate (Amersham) was used as secondary antibody. Peroxidase activity was visualized with an enhanced chemiluminescence detection kit (Pierce). This analysis showed that MV vectors expressed both EDIII tetrameric antigen and NS polyepitopic antigen. Similarly, the pcDNA3-NS plasmid expressed a high level of NS polyepitopic antigen (**Figure 8**).

Mice experiments and cellular immune responses

Mice deficient for IFN α / β receptors and expressing human MV receptor (hCD46+/-IFN α / β -/- or CD46-IFNAR) were produced as previously described

(Combredet *et al.*, *J Virol.*, 2003, 77(21): 11546-11554) and housed under pathogen-free conditions at the Institut Pasteur animal facility. Experiments were conducted following the guidelines of the Office of Laboratory Animal Care at Institut Pasteur. Group of six 6-week-old CD46-IFNAR mice were inoculated *via* the intraperitoneal (ip) route with a single administration of 10^5 TCID₅₀ of MV-DENV vectors or empty MV. Mice were euthanized 7 days post-immunization and spleens were collected. Freshly extracted splenocytes were specifically stimulated for 18h with DENV peptides (2 µg/ml) or MV (MOI 1). Cells were also stimulated by concanavalin A (5 µg/ml, Sigma) as a positive control and by RPMI-IL-2 (10 U/ml) as a negative control. Their capacity to secrete IFN-γ upon stimulation was tested by ELISPOT assay as previously described (Guerbois *et al.*, *Virology*, 2009, 388(1): 191-203).

Peptides

A series of 36 overlapping peptides (9 to 15 amino acids overlapping of 5 amino acids) covering the entire NS polyepitopic sequence were synthesized (**Figure 9**). Five specific peptides were also identified with prediction algorithms able to bind to H2-Db and H2-Kb T cell receptors expressed in CD46-IFNAR mice (**Figure 9**). These peptides were used in ELISPOT experiments either in pools or as individual peptides to restimulate T cell responses in splenocytes from immunized CD46-IFNAR mice.

Immunogenicity in CD46-IFNAR mice

Cell-mediated immune response (CMI) elicited by immunization with MV-DENV was assessed using IFN-γ ELISPOT assay on splenocytes collected 7 days after a single immunization. After *ex vivo* stimulation by DENV NS peptides or MV, a significant number of DENV-specific IFN-γ secreting cells (400-800 spot forming cells/ 10^6 splenocytes) were detected in MV-DENV immunized mice (**Figures 10, 11, 12**), which represented one third of MV-specific response in similar stimulation condition (1500 spot forming cells/ 10^6 splenocytes). Most mice immunized with MV-DENV (5/6) had a significant CMI response to DENV, demonstrating that a single MV-DENV immunization

in this experimental model was able to prime anti-DENV cellular immunity within a week.

Immunogenicity in humanized mice.

5 The pcDNA3 plasmid expressing the DENV1 NS polyepitope sequence under a CMV promoter was injected by electroporation in HLA-A*02:01, HLA-A*24:02, HLA-B*07:02 and HLA-B*35:01 monochain transgenic/ H-2 Class I null mice, and the INF- γ response of spleen cells was quantified by ELISPOT assay against individual peptides (**Figure 13**).

10

Among 47 potential epitopes described to elicit an HLA-restricted T cell response in human donors previously exposed to DENV, 13 epitopes were identified in immunized mice: four in the first domain of NS3, and restricted by HLA-A*02:01, HLA-B*07:02 and HLA-B*35:01, four in the second domain
15 of NS3, restricted by HLA-A*24:02 and HLA-B*35:01, three in NS4b, restricted by HLA-A*24:02 and HLA-B*35:01, and two in NS5 and restricted by HLA-A*02:01 and HLA-B*07:02 (**Figure 14**).

Comparison of the T cell response elicited in the different transgenic mice
20 revealed a higher number of peptides with a higher magnitude in the HLA-B*35:01 mice, in agreement with the high response frequency and magnitude associated with this allele in human donors from hyperendemic area (*Weiskopf D et al., Proc Natl Acad Sci USA 2013, 110(22):E2046-2053*) (**Figure 15**).

25

Interestingly, and in agreement with the conserved antigenic regions selected from the different serotypes (**Figures 1 and 14**), the amino acid sequence of the antigenic epitopes present in the polyepitopic construct was conserved among the other dengue serotypes, in particular for the two anchor residues
30 involved in HLA binding (**Table 3**). This suggested that the CD8⁺ T cells induced by the DENV1-based construct could also recognize peptides derived from DENV2, 3 and 4. This T-cell cross reactivity may be verified

using human EBV-transformed B cell line C1R (*Zemmour J et al., J Immunol 1992, 148(6):1941-1948*) expressing a single HLA allele, and pulsed with the cognate peptide, or infected with DENV1, 2, 3 and 4.

- 5 **Table 3.** Sequence conservation among the 4 DENV serotypes of epitopes of the NS polyepitope sequence identified in mice with 4 different HLA-restrictions. Predicted anchor residues are underlined and highlighted in grey.

A) Peptides HLA-A*02:01

p30	NS polyepitope	R	Y	<u>L</u>	P	A	I	V	R	E	<u>A</u>	I
	Serotype 1	-	-	-	-	-	-	-	-	-	-	-
	Serotype 2	-	-	-	-	-	-	-	-	-	-	-
	Serotype 3	K	-	-	-	-	-	-	-	-	-	-
	Serotype 4	-	I	-	-	S	-	-	-	-	-	L

p451	NS polyepitope	Y	<u>L</u>	P	A	I	V	R	E	<u>A</u>
	Serotype 1	-	-	-	-	-	-	-	-	-
	Serotype 2	-	-	-	-	-	-	-	-	-
	Serotype 3	-	-	-	-	-	-	-	-	-
	Serotype 4	I	-	-	S	-	-	-	-	-

p56	NS polyepitope	S	<u>M</u>	V	N	G	V	V	K	<u>L</u>
	Serotype 1	-	-	-	-	-	-	-	K/R	-
	Serotype 2	-	-	-	-	-	-	-	R/K	-
	Serotype 3	-	-	I	-	-	-	-	-	-
	Serotype 4	-	-	-	-	-	-	-	-	-

B) Peptides HLA-A*24:02

p17	NS poly-epitope	L	D	A	R	T	<u>Y</u>	S	D	P	L	A	L	<u>R</u>	E	F	K	E	F
	Serotype 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Serotype 2	-	-	-	-	I	-	-	-	-	-	-	-	K	-	-	-	-	-
	Serotype 3	K	-	-	-	-	-	-	-	-	-	-	-	K	-	-	-	D	-
	Serotype 4	-	I	-	-	V	-	A	-	-	M	-	-	K	D	-	-	-	-

p32	NS polyepitope	Q	Y	S	D	R	R	W	C	F
	Serotype 1	-	-	-	-	-	-	-	-	-
	Serotype 2	N	-	A	-	-	R/K	-	-	-
	Serotype 3	K	-	T	-	-	K	-	-	-
	Serotype 4	S	-	K	-	-	E	-	-	-

p33	NS polyepitope	I	T	P	M	M	R	H	T	I
	Serotype 1	-	-	-	-	-	-	-	-	-
	Serotype 2	V	-	-	-	L	-	-	S	-
	Serotype 3	-	-	-	-	L	-	-	-	-
	Serotype 4	L	-	-	-	L	-	-	-	-

C) Peptides HLA-B*07:02

p15	NS polyepitope	K	P	R	I	C	T	R	E	E	F
	Serotype 1	-	-	-	-	-	-	-	-	-	-
	Serotype 2	T	-	-	M	-	-	-	K/R	-	-
	Serotype 3	K/R	-	-	L	-	-	H	-	-	-
	Serotype 4	N	-	-	L	-	-	-	-	-	-

p30	NS polyepitope	R	Y	L	P	A	I	V	R	E	A	I
	Serotype 1	-	-	-	-	-	-	-	-	-	-	-
	Serotype 2	-	-	-	-	-	-	-	-	-	-	-
	Serotype 3	K	-	-	-	-	-	-	-	-	-	-
	Serotype 4	-	I	-	-	S	-	-	-	-	-	L

p36	NS polyepitope	A	P	T	R	V	V	A	S	E	M
	Serotype 1	-	-	-	-	-	-	-	-	-	-
	Serotype 2	-	-	-	-	-	-	-	A	-	-
	Serotype 3	-	-	-	-	-	-	-	A	-	-
	Serotype 4	-	-	-	-	-	-	-	A	-	-

D) Peptides HLA-B*35:01

p21	NS polyepitope	H	P	A	S	A	W	T	L	Y
	Serotype 1	H/R	-	-	-	-	-	-	-	-
	Serotype 2	R	-	-	-	-	-	-	-	-
	Serotype 3	-	-	-	-	-	-	-	-	-
	Serotype 4	R	-	-	-	-	-	-	-	-

p49	NS polyepitope	T	<u>P</u>	E	G	I	I	P	A	<u>L</u>
	Serotype 1	-	-	-	-	-	-	-	-	-
	Serotype 2	-	-	-	-	-	-	-	S	<u>M</u>
	Serotype 3	-	-	-	-	-	-	-	-	-
	Serotype 4	-	-	-	-	-	-	-	T	-

p50	NS polyepitope	L	<u>P</u>	V	W	L	S	Y	K	<u>V</u>	A
	Serotype 1	-	-	-	-	-	-	-	-	-	-
	Serotype 2	-	-	-	-	-	A	-	K/R	-	-
	Serotype 3	-	-	-	-	-	A	H	-	-	-
	Serotype 4	-	-	-	-	-	-	-	-	-	-

p51	NS polyepitope	T	<u>L</u>	Y	A	V	A	T	T	<u>I</u>
	Serotype 1	-	-	-	-	-	-	-	-	I/V
	Serotype 2	-	-	-	-	-	-	-	-	F
	Serotype 3	-	-	-	-	-	-	-	-	V
	Serotype 4	-	-	-	-	-	-	-	-	-

p453	NS polyepitope	R	<u>S</u>	W	N	S	G	Y	E	<u>W</u>
	Serotype 1	-	-	-	-	-	-	-	E/D	-
	Serotype 2	-	-	-	-	-	-	H	-	-
	Serotype 3	-	-	-	-	-	-	N	-	-
	Serotype 4	-	-	-	-	T	-	F	D	-

Evidence of an *in vitro* and *in vivo* immune protection induced after DNA immunization with the DENV1-NS polyepitopic construct (SEQ ID NO: 3)

5 A. Induction of DENV-specific cytotoxic T cells in HLA Class I transgenic mice.

To investigate a role of CD8 T cells in a protective immune response, four groups of H-2 Class I null / HLA Class I transgenic mice (HLA-A*02:01, HLA-A*24:02, HLA-B*07:02 and HLA-B*35:01) were vaccinated by DNA
10 immunization. In each group made up of seven transgenic animals, five and two mice received the DENV1-NS polyepitopic construct and the control plasmid, respectively. All animals were immunized by intradermic injection (100 µg DENV1-NS polyepitopic or control plasmid) followed by *in vivo*

electroporation. Two immunizations were performed at three weeks interval, and spleen cells were tested for *in vitro* and *in vivo* cytotoxic activity as well as IFN- γ secretion by ELISPOT assay ten days after the second injection.

5 1- Evaluation of DENV-specific cytotoxic T cell responses *in vitro*.

In each group of HLA Class I transgenic animals, three and one mouse that received the DENV1-NS polyepitopic construct and the control plasmid, respectively, were tested for their antigen-specific cytotoxic T cell responses. Quantification of the T cell response was obtained using the
10 Granzyme B (GrB) ELISPOT assay, which measures at a single cell level the release of the cytotoxic mediator Granzyme B, and which was shown to correlate strongly with the ability of T cells to lyse target cells (Ewen CL et al., 2006, J Immunol Methods, 308(1-2): 156-166; Kalia V et al., 2010, Adv Exp Med Biol, 684: 79-95; Zanetti M, et al. 2010, Adv Exp Med Biol, 684:108-125)
15 and to inhibit viral production (Marcet-Palacios M, et al., 2011, PLoS Pathog, 7(12):e1002447). In this assay, the frequency of spleen cells releasing Granzyme B upon *in vitro* stimulation with cognate or irrelevant peptides was measured in parallel with the frequency of cells secreting IFN- γ . Results showed a significant IFN- γ and GrB response in mice immunized with the
20 DENV1-NS polyepitopic construct, in comparison to the animals immunized with the control pcDNA3.1 plasmid (**Figures 16A and 16B**). There was also a correlation between the IFN- γ response and the release of GrB, with the highest response for both IFN- γ and GrB obtained for peptides P32 in HLA-A24 mice, P56 in HLA-A2 mice, P15 in HLA-B7 mice, and P49, P50 and P51
25 in HLA-B35 mice.

2- Evaluation of DENV-specific cytotoxic T cells *in vivo*.

The *in vivo* cytotoxic assay was performed as previously described (Clemente T. et al., 2013, Methods, 61(2):105-109). Briefly, mice immunized
30 with the DENV1-NS polyepitopic construct or the control plasmid were adoptively transferred with syngeneic cells loaded with peptides and labeled

with a fluorescent dye (Cell trace violet). A mix of 10^7 high- and 10^7 low-fluorescent stained cells, pulsed with specific and control peptides, respectively, was injected intravenously into recipient animals. Eighteen hours after the injection, spleen cells of recipient mice were analyzed by flow cytometry and the ratio between Cell trace high (cells pulsed with a mix of specific peptides) and Cell trace low donor cells (not pulsed or pulsed with control peptides) was determined. As an equivalent number of high and low stained cells were injected into recipient mice, a specific killing activity in immunized mice resulted in a lower fraction of high stained cells loaded with the mix of specific peptides (**Figure 17**). The percentage of specific lysis was determined using the formula:

$$1 - \frac{\% \text{Cell trace violet high immunized} / \% \text{Cell trace violet low immunized}}{\% \text{Cell trace violet high naive} / \% \text{Cell trace violet low naive}} \times 100$$

B. Induction of a protective immune response in humanized mice

Because type I IFN-sufficient mice are resistant to DENV infection, a vaccine-induced protective immune response against a challenge with DENV can only be considered in type I-deficient mice, or in alymphoid mice engrafted with human hematopoietic stem cells. In line with the recent development of new generations of humanized mice reconstituted with human myeloid and lymphoid compartments with HLA-restricted responses (Legrand N, et al., 2011, *Proc Natl Acad Sci U S A*, 108(32): 13224-13229; Garcia S, et al., 2012, *Immunol Lett*, 146(1-2): 1-7; Serra-Hassoun M, et al., 2014, *J Immunol*, 193(3): 1504-1511), the inventors will use RAG^{-/-} γ c^{-/-} mice transgenic for human SIRPalpha, in which mouse MHC class I and class II molecules have been replaced by human HLA-A2 and DR1 molecules, respectively (CH1-2hSa).

In a first setting, HLA-A2 molecules will be in the HHD configuration (with the human α 1 and α 2 domains of HLA-A2 linked to the murine α 3

domain of H-2D^b) (*Pascolo S, et al., 1997, J Exp Med, 185(12): 2043-2051*). The CH1-2hSa HHD mice are currently available. Three months after human hematopoietic cord blood progenitor engraftment, when all the human subsets have been reconstituted in the host, the mice will first be infected with Dengue virus to verify the *in vivo* viral replication in the human dendritic cell compartment. Briefly, two groups of three mice each will be injected intravenously with 10⁵ or 10⁶ PFU of the DENV1 strain KDH0026A (derived from a human clinical isolate). Virus titers will be monitored by quantitative reverse transcription (qRT)-PCR.

10 In a second setting, the HLA-A2 molecules will consist of the full human HHH version of the class I molecule. These new CH1-2hSa HHH hosts, which are currently under development, should improve the binding of the human CD8/TCR molecules to the MHC-peptide complexes allowing a more efficient stimulation of DENV-specific CD8 T cells. Two groups of 6 humanized CH1-2hSa HHH mice each will be immunized with the DENV1-NS polyepitopic and the control plasmid constructs, as described in **Figure 13**. Within each group of 6 reconstituted mice, protection will be assessed by quantifying viral mRNA by qRT-PCR in the blood of infected animals at day 2, 4 and 7 after intravenous injection of 10⁵ or 10⁶ PFU of DENV1 strain KDH0026A within each group of three mice.

C. MVDVax protective efficacy in CD46-IFNAR mice

The inventors evaluated the protective efficacy in CD46-IFNAR mice of recombinant MVDVax vectors expressing a tetravalent DENV antigen composed of the fusion of the envelope domain III (EDIII) of the four DENV serotypes fused to the ectodomain of the membrane protein (ectoM) of DENV1 and expressing simultaneously the DENV1 NS antigen of the invention (described in **Figure 7**). CD46-IFNAR mice were produced as previously described (*Combredet, C. et al., 2003, J Virol, 77(21): 11546-11554*) and housed under pathogen-free conditions at the Institut Pasteur

animal facility. Experiments were conducted following the guidelines of the Office of Laboratory Animal Care at Institut Pasteur. Group of six 6-week-old CD46-IFNAR mice were inoculated *via* the intraperitoneal (ip) route with 10^5 TCID₅₀ of MV-DENV vectors or empty MV. A second administration of the same dose was performed 1 month after priming. To analyze the presence of anti-MV and anti-DENV antibodies, blood was regularly collected by the periorbital route. Two months after the last immunization, all animals were experimentally challenged by intraperitoneal injection of 10^5 TCID₅₀ of mouse-neuro adapted DENV1 Hawaiï strain grown on Vero cells (*Despres et al., J. Virol., 1998, 72(1), 823-829*). After challenge, blood was collected by the periorbital route at day 1, 2, 3, 5, 7 and the animals were followed for clinical signs and weight for the 15 following days. DENV1 genomic viral load was determined in mice sera by one-step qRT-PCR as described below. Sera were heat inactivated at 56°C for 30 min and the presence of anti-MV antibodies was detected using ELISA (Trinity Biotech). HRP-conjugated anti-mouse immunoglobulin (Jackson Immuno Research) was used as secondary antibody. Anti-DENV antibodies were detected as previously described (*Brandler et al., Vaccine, 2010, 28, 6730-6739*) by ELISA using 96-well plates coated with recombinant EDIII proteins from DENV1, DENV2, DENV3, and DENV4 produced in *E. coli* or synthesized *in vitro*. HRP-conjugated anti-mouse immunoglobulin was used as secondary antibody. The endpoint titers of pooled sera were calculated as the reciprocal of the last dilution giving twice the absorbance of sera from MV inoculated mice that served as negative controls.

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D. MVDVax protective efficacy in non-human primates

The inventors will evaluate in non-human primates (NHP) the protective efficacy of recombinant MVDVax vectors.

Experiments will be conducted as follows:

Viruses. Challenge viruses: DENV1 Jamaica strain CVI 1636 3P, isolated in 1977, passages 2 /3 MP/ 3P C6/36HT 2P Vero cells culture supernatant. DENV1 strain KDH0026A, C6/36 cell culture supernatant. DENV2 DJ.M.O.1.7.12, C6/36 cell culture supernatant. DENV4 Dominica
5 814669 strain, isolated in 1981, passages 4P C6/36 HT 2P Vero cells culture supernatant. These viruses are used for neutralization tests. The recombinant MVDVax vaccine virus is prepared as previously described (*Brandler et al., Vaccine, 2010, 28, 6730-6739*) and viral titer is determined by endpoint limit-dilution assay on Vero cells.

10 **Animals.** Mauritian-derived cynomolgus macaques (*Macaca fascicularis*) are housed in BSL-3 animal care facility at the CEA. The Ile-de-France region ethics committee will approve experimental methods that are conducted in accordance with the European Directive 2010-63-UE. The animals are juvenile male and female adults (0.8–1 kg). To evaluate the
15 immunogenicity of the vaccine candidate, 12 animals are inoculated subcutaneously with two 200- μ L doses containing 10^5 TCID₅₀ of MVDvax vaccine virus one month apart and are boosted six month later with a third dose of 10^6 TCID₅₀ of MVDVax. Similarly, eight control animals receive two administrations of empty MVSchw vaccine (200 μ L containing 10^5 TCID₅₀)
20 and a third dose of 10^6 TCID₅₀ six months later. Five months after the last immunization, the animals are divided in two groups and challenged by intravenous inoculation of 1.0×10^4 pfu of wild type DENV1 or DENV4 suspended in 200 μ L PBS. Monkeys are monitored for viremia, clinical signs, and cellular and antibody response. Peripheral blood mononuclear cells
25 (PBMC) are isolated by Percoll density gradient centrifugation (Sigma-Aldrich). Serum and plasma are collected and stored at -20°C for later analysis.

Humoral responses. Anti-DENV and anti-MV antibodies are detected in heat-inactivated sera by use of an enzyme-linked immunosorbant assay
30 (ELISA) as previously described (*Brandler et al., Vaccine, 2010, 28, 6730-*

6739). Sera are considered positive when the optical density (OD) is twice the OD of sera from control animals.

DENV neutralization test. Anti-DENV neutralizing antibodies are detected using a focus reduction neutralization test (PRNT) in Vero cells as previously described (*Brandler et al., PLoS 2007, 1(3), e96*). Briefly, anti-DENV neutralizing antibodies are detected on Vero cells using 50 FFU of Vero-adapted DENV1 Hawaiï strain (WHO reference strain, Genbank accession no. AF226687), DENV2 Jamaica strain N.1409, DENV3 strain PaH881/88 Thailand, or DENV4 strain 63632/76 Burma. The endpoint titer is calculated as the highest serum dilution that reduces the number of FFU by at least 50%. The neutralizing antibody titer is also tested at the Center for Vaccine Development (CVD, Mahidol University, Thailand). In the tests conducted at the CVD, monkey kidney-derived LLC-MK2 cells and the following DENV are used: DENV1 (16007), DENV2 (16681), DENV3 (16562), and DENV4 (1036), and the PRNT titer is calculated based on a 50% reduction in plaque count (PRNT50) as previously described (*Sirivichayakul et al. Virol. J. 2014, 11-48*).

Cellular immune responses. Cellular responses are detected by Elispot assay and by polyfunctional flow cytometry following stimulation of lymphocytes from peripheral blood with synthetic peptide pools covering the DENV1-NS insert of MVDVax, as previously described (*Stebbing et al, PLoS-One, 2012, 7(11), e50397*). Cells are stimulated in triplicate with synthetic 15-mer (overlapping by 11aa) peptide pools (*BEI, <http://www.beiresources.org>*) at 1 µg/ml/peptide or a live-attenuated empty MV at 10^4 TCID₅₀ / 10^6 cells, in the presence of 1 µg/ml of the co-stimulatory antibodies anti-CD28 and CD49d (Biolegend). Negative controls are incubated with an equal volume of DMSO (0.15% v/v) without peptide and positive controls with 1 µg/ml Staphylococcal Enterotoxin B (Sigma-Aldrich).

Quantification of DENV viral RNA and DENV titration. Sera (25 µL) are diluted in 0.5 mL Dulbecco's modified Eagle's medium (DMEM)/5% fetal calf serum (FCS). Viral RNA is extracted (QIAamp viral RNA extraction kit,

Qiagen) and analyzed by one step DENV qRT-PCR, using high fidelity enzymes (Roche, Mannheim, Germany). Real-time qRT-PCR of DENV RNA is performed with RealArt™ WNV LC real-time PCR kit (Qiagen). For virus titration, samples are diluted in 250 µL of medium, and infectivity of serial
5 dilutions is assayed on Vero cells overlaid with DMEM Glutamax/2% FCS containing 0.8% final (weight/volume) carboxy methylcellulose. After 4 days of incubation, cells are fixed and plaques are visualized by an immuno-focus assay as previously described (*Brandler et al., PLoS 2007, 1(3), e96*).

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1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
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1-3	Identification of deposit	
1-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
1-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
1-3-3	Date of deposit	12 June 2002 (12.06.2002)
1-3-4	Accession Number	CNCM I-2889
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2-3-3	Date of deposit	14 June 2006 (14.06.2006)
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3-3-1	Name of depositary institution	CNCM Collection nationale de cultures de microorganismes (CNCM)
3-3-2	Address of depositary institution	Institut Pasteur, 28, Rue du Docteur Roux, 75724 Paris Cédex 15, France
3-3-3	Date of deposit	04 August 2006 (04.08.2006)
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CLAIMS

1. A chimeric polyepitope having less than 600 amino acid residues comprising or consisting of the following fragments of (a), (b) and (c) assembled in a fusion polypeptide wherein the fragments of (a), (b) and (c) are directly or indirectly fused in this order:

(a) two fragments of the non-structural (NS) NS3 protein of the dengue virus (DENV) serotype 1 (DENV1) comprising or consisting of two regions, wherein the first region has an amino acid sequence as defined in SEQ ID NO: 6, and the second region has an amino acid sequence as defined in SEQ ID NO: 9,

(b) a fragment of the NS4b protein of DENV1 having an amino acid sequence as defined in SEQ ID NO: 12,

(c) a fragment of the NS5 protein of DENV1 having an amino acid sequence as defined in SEQ ID NO: 15,

or a variant of the chimeric polyepitope having an amino acid sequence which has more than 90% identity with the sequence of the chimeric polyepitope over its whole length,

wherein the chimeric polyepitope elicits a human leukocyte antigen (HLA)-restricted CD8⁺ and/or CD4⁺ T cell response against DENV1, DENV2, DENV3 and DENV4.

2. The chimeric polyepitope according to claim 1, which has an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 146, SEQ ID NO: 147 and SEQ ID NO: 148.

3. An isolated or purified polynucleotide encoding the chimeric polyepitope according to claim 1 or 2.

4. The polynucleotide according to claim 3, which has a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 or SEQ ID NO: 2.

5. A vector, which is a plasmid comprising the polynucleotide according to claim 3 or 4.

6. A recombinant measles virus (MV) genome vector, which is a plasmid comprising (i) a cDNA encoding the full-length RNA genome of a MV virus and (ii) the polynucleotide according to claim 3 or 4, said polynucleotide being located as an insert between the P and M genes of the MV genome or between the H and L genes of the MV genome, optionally in an Additional Transcription Unit (ATU), wherein the sequences of (i) and (ii) when recombined in the plasmid together comply with the rule of six.

7. A recombinant MV genome vector, which is a plasmid comprising (a) a cDNA encoding the full-length RNA genome of a MV virus and (b) a polynucleotide encoding DENV antigens composed of the fusion of the envelope domain III (EDIII) polypeptides of the four DENV serotypes, fused to the ectodomain of the membrane protein (ectoM) of DENV1, in particular DENV antigens having the sequence of SEQ ID NO: 145 and (c) the polynucleotide according to claim 3 or 4, wherein:

- the polynucleotide (b) is located as an insert between the P and M genes of the MV genome and the polynucleotide (c) is located as an insert between the H and L genes of the MV genome, or

- the polynucleotide (b) is located as an insert between the P and M genes of the MV genome and the polynucleotide (c) is located as an insert between the P and M genes of the MV genome, or

- the polynucleotide (b) is located as an insert between the H and L genes of the MV genome and the polynucleotide (c) is located as an insert between the P and M genes of the MV genome and

wherein the sequences of (a), (b) and (c) when recombined in the plasmid together comply with the rule of six.

8. The recombinant MV genome vector according to claim 6 or 7, which comprises the cDNA encoding the full-length RNA genome of a live-attenuated Schwarz or Moraten virus.

9. A host cell genetically transformed with the polynucleotide according to claim 3 or 4, the vector according to claim 5, or the recombinant MV genome vector according to any one of claims 6 to 8.

10. The cell according to claim 9, which is HEK-293-T7-MV cell line.

11. Recombinant measles virus (MV) particles, which are rescued from a helper cell line expressing the T7 RNA polymerase and the N, P and L proteins of MV, and which further expresses a nucleic acid encoding a vector according to claim 5 or a nucleic acid encoding a recombinant MV genome vector according to any one of claims 6 to 8.

12. An immunogenic composition comprising (a) DENV antigens composed of the fusion of the EDIII polypeptides of the four DENV serotypes, fused to ectoM of DENV1, in particular DENV antigens having the sequence of SEQ ID NO: 145 and (b) the chimeric polyepitope according to claim 1 or 2, which optionally does not comprise an accessory adjuvant.

13. An immunogenic composition comprising recombinant MV particles according to claim 11, which optionally does not comprise an accessory adjuvant.

14. The immunogenic composition according to claim 12 or 13, wherein said composition is formulated for an administration through parenteral route

such as subcutaneous (s.c.), intradermal (i.d.), intramuscular (i.m.), intraperitoneal (i.p.) or intravenous (i.v.) injection.

15. The immunogenic composition according to any one of claims 12 to 14, wherein said composition is to be administered in one or multiple administration dose(s), in particular in a prime-boost administration regime.

16. The use of the recombinant MV particles according to claim 11 or the immunogenic composition according to claim 12, in the manufacture of a medicament for the prevention and/or treatment of a dengue virus infection in a human subject.

17. A method to produce recombinant MV particles for the preparation of an anti-dengue virus vaccine, comprising or consisting of:

a) transfecting a recombinant MV genome vector according to any one of claims 6 to 8, in a helper cell line which expresses the T7 RNA polymerase and the N, P and L proteins of MV;

b) transferring said helper cell line onto cells competent to sustain the replication and production of recombinant MV particles expressing a chimeric polyepitope according to claim 1 or 2 and optionally a polypeptide comprising a tetravalent DENV antigen composed of the fusion of the EDIII polypeptides of the four serotypes, fused to ectoM of DENV1, in particular DENV antigens having the sequence of SEQ ID NO: 145 and;

c) recovering the recombinant MV particles produced in step b).

18. The method according to claim 17, wherein said competent cells in step b) are Vero (African green monkey kidney) cells, CEF (chick embryo fibroblast) cells or MRC5 cells.