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(72) Inventeur/Inventor:  
LANZAVECCHIA, ANTONIO, CH  
(73) Propriétaire/Owner:  
INSTITUTE FOR RESEARCH IN BIOMEDICINE, CH  
(74) Agent: MBM INTELLECTUAL PROPERTY LAW LLP

(54) Titre : ANTICORPS DE NEUTRALISATION DU VIRUS DE LA DENGUE ET LEURS UTILISATIONS  
(54) Title: DENGUE VIRUS NEUTRALIZING ANTIBODIES AND USES THEREOF

(57) **Abrégé/Abstract:**

The invention relates to antibodies and antigen binding fragments thereof and to cocktails of antibodies and antigen binding fragments that neutralize dengue virus infection without contributing to antibody-dependent enhancement of dengue virus infection. The invention also relates to immortalized B cells that produce, and to epitopes that bind to, such antibodies and antigen binding fragments. In addition, the invention relates to the use of the antibodies, antigen binding fragments, and epitopes in screening methods as well as in the diagnosis and therapy of dengue virus infection.

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(71) Applicant (for all designated States except US): **INSTITUTE FOR RESEARCH IN BIOMEDICINE** [CH/CH]; Via Vela 6, CH-5400 Bellinzona (CH).

## (72) Inventor; and

(75) Inventor/Applicant (for US only): **LANZAVECCHIA, Antonio** [CH/CH]; Institute For Research In Biomedicine, Via Vela 6, CH-5400 Bellinzona (CH).

(74) Agent: **SMAGGASGALE, Gillian, Helen**; W.P. Thompson & Co., 55 Drury Lane, London WC2B 5SQ (GB).

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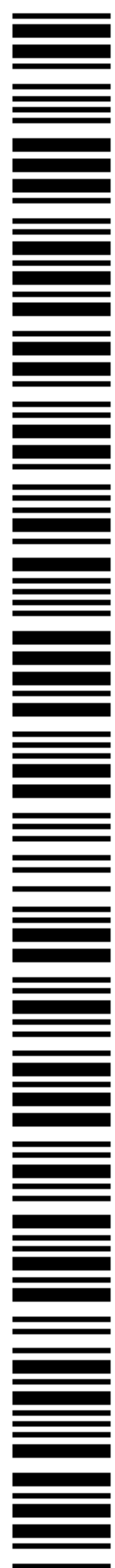
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(54) Title: DENGUE VIRUS NEUTRALIZING ANTIBODIES AND USES THEREOF

(57) Abstract: The invention relates to antibodies and antigen binding fragments thereof and to cocktails of antibodies and antigen binding fragments that neutralize dengue virus infection without contributing to antibody-dependent enhancement of dengue virus infection. The invention also relates to immortalized B cells that produce, and to epitopes that bind to, such antibodies and antigen binding fragments. In addition, the invention relates to the use of the antibodies, antigen binding fragments, and epitopes in screening methods as well as in the diagnosis and therapy of dengue virus infection.



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## DENGUE VIRUS NEUTRALIZING ANTIBODIES AND USES THEREOF

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### BACKGROUND

Dengue viruses (DENV) are human pathogens with a significant threat to world health. These viruses are estimated to cause several hundred thousand cases of dengue fever, dengue hemorrhagic fever and dengue shock syndrome annually. There are four closely related serotypes of dengue viruses, DENV-1, DENV-2, DENV-3 and DENV-4, of the genus *Flavivirus*.  
 10 The four viruses are spread from human to human through the bite of *Aedes aegypti*, a highly urbanized mosquito species that has successfully resisted all attempts at eradication and control. Vaccination is considered to be the only efficient method of control of dengue. To this end, several tetravalent dengue candidate vaccines are in late stages of development.

A first infection with one Dengue virus serotype induces a life-long protective immunity  
 15 to the homologous serotype. However, there is no cross-protection against infection by a different serotype. Indeed, pre-existing immunity against one serotype is associated with increased risk for dengue infection and dengue hemorrhagic fever caused by a different serotype due to antibody-dependent enhancement (ADE) of infection. In ADE, antibodies raised by prior dengue infection or passively transferred from mother form infectious immune complexes that  
 20 attach to Fc-receptor-bearing cells in the mononuclear phagocyte lineage resulting in efficient infection.

Accordingly, there is a need for materials and methods for preventing dengue virus infection without increasing the risk of antibody-dependent enhancement of infection.

### SUMMARY

25 The invention is based, in part, on the discovery of antibodies and cocktails of antibodies that neutralize dengue virus infection without contributing to antibody-dependent enhancement of dengue virus infection. Accordingly, in one aspect of the invention, the invention comprises a human antibody, an antibody variant, or an antigen binding fragment thereof, that neutralize a dengue virus, wherein the antibody, antibody variant, or antigen binding fragment does not  
 30 contribute to antibody-dependent enhancement of dengue virus infection. In one embodiment,

the invention comprises a human antibody, an antibody variant, or an antigen binding fragment thereof, that neutralize a dengue virus, wherein the antibody, antibody variant, or antigen binding fragment comprises a mutation in the Fc region, and wherein the mutation reduces binding of the antibody to an Fc receptor.

5           In another embodiment of the invention, the invention comprises a pharmaceutical composition comprising two or more human antibodies, or antigen binding fragments thereof. The antibodies or antigen binding fragments neutralize dengue virus serotypes DENV-1, DENV-2, DENV-3, and DENV-4 by binding at least two distinct epitopes on each dengue virus serotype. The antibodies of the pharmaceutical composition do not contribute to antibody-  
10           dependent enhancement of dengue virus infection.

          In yet another embodiment, the invention comprises an antibody, or an antigen binding fragment thereof, comprising at least one complementarity determining region (CDR) sequence having the sequence of any one of SEQ ID NOs: 1-6, 17-22, 33-38, 49-54, 67-72, 83-88, 99, 100, 105-110, 121-123, 124, 125, 135-139, 149, 153-158, 169-174, 185-188, or 189, wherein the  
15           antibody neutralizes dengue virus infection.

          In yet another embodiment, the invention comprises an antibody, or an antigen binding fragment thereof, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or a heavy chain variable region comprising the amino acid  
20           sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 46; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 61 and a light chain variable region comprising the amino acid  
25           sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 65 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 79 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; or a heavy chain variable region comprising the amino acid  
30           sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 96; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 103; or a heavy chain variable region comprising the amino acid

sequence of SEQ ID NO: 117 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 118; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 131 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 132; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 151 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 165 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 166; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 182; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 195 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 196, wherein the antibody neutralizes dengue virus infection.

15 In a further embodiment, the invention comprises a recombinant antibody, antibody variant, or antigen binding fragment thereof, that can neutralize a dengue virus. The recombinant antibody, antibody variant, or antigen binding fragment does not contribute to antibody-dependent enhancement of dengue virus infection.

20 In another aspect, the invention comprises a nucleic acid molecule comprising a polynucleotide encoding an antibody or antibody fragment of the invention that neutralizes dengue virus infection. In yet another aspect, the invention comprises a cell expressing an antibody of the invention. In still another aspect, the invention comprises an isolated or purified immunogenic polypeptide comprising an epitope that binds to an antibody of the invention.

25 The invention also comprises a pharmaceutical composition comprising an antibody, an antibody variant or an antigen binding fragment of the invention, a nucleic acid of the invention, or an immunogenic polypeptide of the invention and a pharmaceutically acceptable diluent or carrier and, optionally, an agent useful for extending the half life of the antibody or antigen binding fragment thereof.

30 In another aspect of the invention, the invention provides a method of inhibiting or preventing dengue virus infection or a dengue virus-related disease or a method of treating dengue virus infection or a dengue virus-related disease. The method comprises administering to a subject in need thereof, a therapeutically effective amount of at least one antibody, antibody variant, antigen binding fragment, or a pharmaceutical composition of the invention.

In yet another aspect of the invention, the invention comprises a method of screening for polypeptides that can induce or reveal an immune response against dengue virus, comprising screening polypeptide libraries using an antibody, an antibody fragment or variant of the invention.

5 In yet another aspect of the invention, the invention comprises a method of monitoring the quality of anti-dengue virus vaccines. The method comprises using an antibody, an antibody variant, or an antigen binding fragment thereof of the invention to check that the antigen of the vaccine contains the specific epitope in the correct conformation.

10 In a further aspect of the invention, the invention comprises a vaccine comprising an epitope which specifically binds to an antibody, an antibody fragment or variant of the invention.

Use of an antibody of the invention, or an antigen binding fragment thereof, a nucleic acid of the invention, an immunogenic polypeptide of the invention, or a pharmaceutical composition of the invention (i) in the manufacture of a medicament for the treatment of dengue virus infection, (ii) in a vaccine, or (iii) in diagnosis of dengue virus infection is also  
15 contemplated to be within the scope of the invention. Further, use of an antibody of the invention, or an antigen binding fragment thereof, for monitoring the quality of anti-DENV vaccines by checking that the antigen of said vaccine contains the specific epitope in the correct conformation is also contemplated to be within the scope of the invention.

In a further aspect, the invention comprises an epitope which specifically binds to an  
20 antibody of any one of the invention, or an antigen binding fragment thereof, for use (i) in therapy, (ii) in the manufacture of a medicament for treating dengue virus infection, (iii) as a vaccine, or (iv) in screening for ligands able to neutralise dengue virus infection.

### BRIEF DESCRIPTION OF FIGURES

Figure 1. VERO cells and K562 cells were used in virus neutralization and enhancement  
25 assays with each serotype of dengue virus using wild-type anti-dengue virus antibodies. Antibodies to dengue virus inhibit infection of the target virus on VERO cells in a dose-dependent manner. On K562 cells, the antibodies lead to a dose dependent antibody-dependent enhancement (ADE) of infection.

Figure 2. Anti-dengue virus antibodies that have a CH2 L4A and L5A substitution  
30 (LALA variants) in the heavy chain neutralize target virus infection on VERO cells as did the unmodified antibodies. However, the LALA variants completely abolished the antibody-dependent enhancement of infection by the target virus on K562 cells.

## DETAILED DESCRIPTION OF THE INVENTION

The invention is based on the discovery of antibodies and cocktails of antibodies that neutralize dengue virus (DENV) infection without contributing to antibody-dependent enhancement (ADE) of dengue virus infection. In one aspect of the invention, the invention  
5 comprises a human antibody, a variant antibody, or an antigen binding fragment thereof, that neutralizes a dengue virus without contributing to antibody-dependent enhancement of dengue virus infection. The antibodies or antibody fragments can neutralize more than one dengue virus serotype, for example, 2, 3 or all 4 dengue virus serotypes DENV-1, DENV-2, DENV-3, and DENV-4.

10 The invention also comprises a pharmaceutical composition comprising, for example, an antibody cocktail that comprises two or more human antibodies, antibody variants or antigen binding fragments thereof. The pharmaceutical compositions of the invention comprising a cocktail of human antibodies, antibody fragments or variants neutralize all four dengue virus serotypes, *i.e.*, DENV-1, DENV-2, DENV-3, and DENV-4. In one embodiment, the cocktail of  
15 antibodies, antibody fragments or variants neutralize dengue virus by binding at least two distinct epitopes on each dengue virus serotype. It is noted that the antibodies, variants and fragments of the pharmaceutical composition do not contribute to antibody-dependent enhancement of dengue virus infection. In one embodiment, the cocktail comprises two antibodies, fragments or variants thereof. In another embodiment, the cocktail comprises three antibodies, fragments or variants  
20 thereof. In yet another embodiment, the cocktail comprises more than 3 antibodies, *e.g.*, 4, 5, 6, 7 or 8 antibodies.

As used herein, the terms “fragment,” “antibody fragment,” and “antigen binding fragment” are used interchangeably to refer to any fragment of an antibody of the invention that retains the antigen-binding activity of the antibody. Exemplary antibody fragments include, but  
25 are not limited to, Fab, Fab’, F(ab’)<sub>2</sub>, Fv, and scFv fragments.

The terms “mutation,” and “substitution” are used interchangeably to refer to a change in one or more nucleic acid or amino acid residues.

As used herein, the terms “variant,” and “antibody variant” are used interchangeably to refer to any variant of an antibody of the invention that retains the antigen-binding activity of the  
30 antibodies. The term variant includes antibodies that comprise mutations and/or substitutions. Exemplary antibody variants include, but are not limited to, those that have an L to A substitution at position CH2 4, 5, or both.

**Antibodies of the invention**

The invention provides antibodies that neutralize dengue virus, but do not contribute to ADE of dengue virus infection. A “neutralizing antibody” is one that can neutralize the ability of a pathogen to initiate and/or perpetuate an infection in a host. The antibodies of the invention  
5 are able to neutralize one or more dengue virus serotypes DENV-1, DENV-2, DENV-3, and DENV-4. In one embodiment, the antibody of the invention neutralizes more than one, *e.g.*, 2, 3, or all 4 dengue virus serotypes. In another embodiment, a pharmaceutical composition comprising two or more antibodies, antibody fragments or variants can neutralize all 4 dengue virus serotypes. In yet another embodiment, the pharmaceutical composition comprising two or  
10 more antibodies, antibody fragments or variants neutralizes dengue virus infection by targeting two distinct epitopes on each dengue virus serotype. These antibodies, antigen binding fragment and variants can be used as prophylactic or therapeutic agents upon appropriate formulation, or as a diagnostic tool, as described herein.

The antibodies of the invention may be monoclonal, for example, human monoclonal  
15 antibodies, or recombinant antibodies. The invention also provides fragments of the antibodies of the invention, particularly fragments that retain the antigen-binding activity of the antibodies. Although the specification, including the claims, may, in some places, refer explicitly to antibody fragment(s), variant(s) and/or derivative(s) of antibodies, it is understood that the term “antibody” or “antibody of the invention” includes all categories of antibodies, namely, antibody  
20 fragment(s), variant(s) and derivative(s) of antibodies.

Without being bound to any theory, it is believed that antibody-dependent enhancement of dengue virus infection is brought about by the binding of the Fc region of the antibody, in particular, the Fc region of the heavy chain of an IgG molecule, to an Fc receptor, *e.g.*, an Fcγ receptor on a host cell. The invention, on the other hand, provides antibodies, including IgG  
25 molecules, that have reduced binding to the Fc receptors (FcR). In one embodiment, the antibody of the invention comprises one or more mutations in the Fc region. The mutation(s) may be any mutation that reduces binding of the antibody to an Fc receptor. In one embodiment, the Fc region of an antibody of the invention comprises a substitution at positions CH2 4, 5, or both. In general, the amino acid at positions 4 and 5 of CH2 of the wild-type IgG1 and IgG3 is a  
30 leucine (“L”). In one embodiment, the antibodies of the invention comprise an amino acid at position CH2 4, 5, or both, that is not an L. In another embodiment, the antibodies of the invention comprise an alanine (“A”) at position CH2 4, or 5, or both. An antibody comprising a CH2 L4A and an L5A substitution is referred to herein as a “LALA” variant.

Alternatively, the invention provides antibody fragments that do not comprise an Fc region and thus do not bind to an FcR. Exemplary antibody fragments include, but are not limited to, Fab, Fab', F(ab')<sub>2</sub>, Fv and scFv.

The sequences of the heavy chains and light chains of several exemplary antibodies of the invention, each comprising three CDRs on the heavy chain and three CDRs on the light chain have been determined. The position of the CDR amino acids are defined according to the IMGT numbering system [1, 2, 3]. The sequences of the CDRs, heavy chains, light chains as well as the sequences of the nucleic acid molecules encoding the CDRs, heavy chains, light chains of many exemplary antibodies of the invention are disclosed in the sequence listing. Table 1 provides the SEQ ID NOs for the amino acid sequences of the six CDRs, the variable region of the heavy and light chains, respectively, of exemplary antibodies of the invention. Table 2 provides the SEQ ID NOs for the sequences of the nucleic acid molecules encoding the CDRs, heavy chains and light chains of exemplary antibodies of the invention.

Table 1. Amino Acid SEQ IDs for Antibody CDRs, Heavy and Light Chains

| Antibody  | CDRs                 | Heavy Chain Variable Region | Light Chain Variable Region |
|-----------|----------------------|-----------------------------|-----------------------------|
| HMB-DV-1  | 1-6                  | 13                          | 14                          |
| HMB-DV-2  | 17-22                | 29                          | 30                          |
| HMB-DV-3  | 33-38                | 45                          | 46                          |
| HMB-DV-4  | 49-54                | 61, 65                      | 62                          |
| HMB-DV-5  | 67-72                | 79                          | 80                          |
| HMB-DV-6  | 83-88                | 95                          | 96                          |
| HMB-DV-7  | 83-85, 99, 53, 100   | 95                          | 103                         |
| HMB-DV-8  | 105-110              | 117                         | 118                         |
| HMB-DV-9  | 121-123, 70 124, 125 | 131                         | 132                         |
| HMB-DV-10 | 135-139, 109         | 145                         | 146                         |
| HMB-DV-11 | 149, 136-139, 109    | 151                         | 146                         |
| HMB-DV-12 | 153-158              | 165                         | 166                         |
| HMB-DV-13 | 169-174              | 181                         | 182                         |
| HMB-DV-14 | 185-188, 37, 189     | 195                         | 196                         |

Table 2. Nucleic Acid SEQ IDs for Antibody CDRs, Heavy and Light Chains

| Antibody | CDRs                | Heavy Chain Variable Region | Light Chain Variable Region |
|----------|---------------------|-----------------------------|-----------------------------|
| HMB-DV-1 | 7-12                | 15                          | 16                          |
| HMB-DV-2 | 23-28               | 31                          | 32                          |
| HMB-DV-3 | 39-44               | 47                          | 48                          |
| HMB-DV-4 | 55-60               | 63, 66                      | 64                          |
| HMB-DV-5 | 73-78               | 81                          | 82                          |
| HMB-DV-6 | 89-94               | 97                          | 98                          |
| HMB-DV-7 | 89-91, 101, 59, 102 | 97                          | 104                         |
| HMB-DV-8 | 111-116             | 119                         | 120                         |

|           |                        |     |     |
|-----------|------------------------|-----|-----|
| HMB-DV-9  | 126-128, 76, 129, 130  | 133 | 134 |
| HMB-DV-10 | 140-143, 115, 144      | 147 | 148 |
| HMB-DV-11 | 150, 141-143, 115, 144 | 152 | 148 |
| HMB-DV-12 | 159-164                | 167 | 168 |
| HMB-DV-13 | 175-180                | 183 | 184 |
| HMB-DV-14 | 190-193, 43, 194       | 197 | 198 |

In one embodiment, the antibodies or antigen-binding fragments of the invention comprise one or more heavy or light chain CDRs of the exemplary antibodies of the invention. In an exemplary embodiment, the antibodies or antigen-binding fragments of the invention neutralize dengue virus infection and comprise at least one CDR sequence having the sequence  
5 of any one of SEQ ID NOs: 1-6, 17-22, 33-38, 49-54, 67-72, 83-88, 99, 100, 105-110, 121-123, 124, 125, 135-139, 149, 153-158, 169-174, 185-188, or 189.

In another embodiment, the antibodies, antibody variants or antigen binding fragments of the invention comprise a heavy chain comprising an amino acid sequence of one or more of SEQ ID NOs: 1-3, 17-19, 33-35, 49-51, 67-69, 83-85, 105-107, 121-123, 135-137, 149, 153-155, 169-  
10 171, or 185-187. In yet another embodiment, the antibodies, antibody variants or antigen binding fragments of the invention comprise a heavy chain CDR1 selected from the group consisting of SEQ ID NOs: 1, 17, 33, 49, 67, 83, 105, 121, 135, 149, 153, 169, and 185; a heavy chain CDR2 selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 68, 84, 106, 122, 136, 154, 170, and 186; and a heavy chain CDR3 selected from the group consisting of SEQ ID NOs: 3,  
15 19, 35, 51, 69, 85, 107, 123, 137, 155, 171, and 187.

For example, the antibodies of the invention comprise a heavy chain comprising SEQ ID NO: 1 for CDRH1, SEQ ID NO: 2 for CDRH2, SEQ ID NO: 3 for CDRH3; SEQ ID NO: 17 for CDRH1, SEQ ID NO: 18 for CDRH2 and SEQ ID NO: 19 for CDRH3; SEQ ID NO: 33 for CDRH1, SEQ ID NO: 34 for CDRH2 and SEQ ID NO: 35 for CDRH3; SEQ ID NO: 49 for  
20 CDRH1, SEQ ID NO: 50 for CDRH2 and SEQ ID NO: 51 for CDRH3; SEQ ID NO: 67 for CDRH1, SEQ ID NO: 68 for CDRH2 and SEQ ID NO: 69 for CDRH3; SEQ ID NO: 83 for CDRH1, SEQ ID NO: 84 for CDRH2 and SEQ ID NO: 85 for CDRH3; SEQ ID NO: 105 for CDRH1, SEQ ID NO: 106 for CDRH2 and SEQ ID NO: 107 for CDRH3; SEQ ID NO: 121 for CDRH1, SEQ ID NO: 122 for CDRH2 and SEQ ID NO: 123 for CDRH3; SEQ ID NO: 135 for  
25 CDRH1, SEQ ID NO: 136 for CDRH2 and SEQ ID NO: 137 for CDRH3; SEQ ID NO: 149 for CDRH1, SEQ ID NO: 136 for CDRH2 and SEQ ID NO: 137 for CDRH3; SEQ ID NO: 153 for CDRH1, SEQ ID NO: 154 for CDRH2 and SEQ ID NO: 155 for CDRH3; SEQ ID NO: 169 for CDRH1, SEQ ID NO: 170 for CDRH2 and SEQ ID NO: 171 for CDRH3; and SEQ ID NO: 185 for CDRH1, SEQ ID NO: 186 for CDRH2 and SEQ ID NO: 187 for CDRH3.

In yet another embodiment, the antibodies, antibody variants or antibody fragments of the invention comprise a light chain comprising an amino acid sequence of one or more of SEQ ID NOs: 4-6, 20-22, 36-38, 52-54, 70-72, 86-88, 99, 100, 108-110, 124, 125, 138, 139, 156-158, 172-174, 188, or 189. In a further embodiment, the antibodies, antibody variants or antibody

5 fragments of the invention comprise a light chain CDR1 selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 70, 86, 99, 108, 138, 156, 172, and 188; a light chain CDR2 selected from the group consisting of SEQ ID NOs: 5, 21, 37, 53, 71, 87, 109, 124, 157, and 173; and a light chain CDR3 selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 72, 88, 100, 110, 125, 139, 158, 174, and 189.

10 For example, the antibodies of the invention comprise a light chain comprising SEQ ID NO: 4 for CDRL1, SEQ ID NO: 5 for CDRL2; SEQ ID NO: 6 for CDRL3; SEQ ID NO: 20 for CDRL1, SEQ ID NO: 21 for CDRL2; SEQ ID NO: 22 for CDRL3; SEQ ID NO: 36 for CDRL1, SEQ ID NO: 37 for CDRL2; SEQ ID NO: 38 for CDRL3; SEQ ID NO: 52 for CDRL1, SEQ ID NO: 53 for CDRL2; SEQ ID NO: 54 for CDRL3; SEQ ID NO: 70 for CDRL1, SEQ ID NO: 71

15 for CDRL2; SEQ ID NO: 72 for CDRL3; SEQ ID NO: 86 for CDRL1, SEQ ID NO: 87 for CDRL2; SEQ ID NO: 88 for CDRL3; SEQ ID NO: 99 for CDRL1, SEQ ID NO: 53 for CDRL2; SEQ ID NO: 100 for CDRL3; SEQ ID NO: 108 for CDRL1, SEQ ID NO: 109 for CDRL2; SEQ ID NO: 110 for CDRL3; SEQ ID NO: 70 for CDRL1, SEQ ID NO: 124 for CDRL2; SEQ ID NO: 125 for CDRL3; SEQ ID NO: 138 for CDRL1, SEQ ID NO: 109 for CDRL2; SEQ ID NO:

20 139 for CDRL3; SEQ ID NO: 156 for CDRL1, SEQ ID NO: 157 for CDRL2; SEQ ID NO: 158 for CDRL3; SEQ ID NO: 172 for CDRL1, SEQ ID NO: 173 for CDRL2; SEQ ID NO: 174 for CDRL3; and SEQ ID NO: 188 for CDRL1, SEQ ID NO: 37 for CDRL2; SEQ ID NO: 189 for CDRL3.

In one embodiment, an antibody of the invention, or antigen binding fragment thereof,

25 comprises all of the CDRs of antibody HMB-DV-1 as listed in Table 1, and neutralizes dengue virus infection in a human host. In another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-2 as listed in Table 1, and neutralizes dengue virus infection in a human host. In another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-

30 DV-3 as listed in Table 1, and neutralizes dengue virus infection in a human host. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-4 as listed in Table 1, and neutralizes dengue virus infection in a human host. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-5 as listed in Table 1, and

neutralizes dengue virus infection in a human host. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-6 as listed in Table 1, and neutralizes dengue virus infection in a human host. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-7 as listed in Table 1, and neutralizes dengue virus infection in a human host.

In a further embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-8 as listed in Table 1, and neutralizes dengue virus infection in a human host. In another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-9 as listed in Table 1, and neutralizes dengue virus infection in a human host. In still another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-10 as listed in Table 1, and neutralizes dengue virus infection in a human host. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-11 as listed in Table 1, and neutralizes dengue virus infection in a human host. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-12 as listed in Table 1, and neutralizes dengue virus infection in a human host. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-13 as listed in Table 1, and neutralizes dengue virus infection in a human host. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody HMB-DV-14 as listed in Table 1, and neutralizes dengue virus infection in a human host.

In still another embodiment, the antibodies of the invention comprise a heavy chain with an amino acid sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to those of SEQ ID NOs: 13, 29, 45, 61, 65, 79, 95, 117, 131, 145, 151, 165, 181, or 195. In yet another embodiment, the antibodies of the invention comprise a light chain with an amino acid sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to those of SEQ ID NOs: 14, 30, 46, 62, 80, 96, 103, 118, 132, 146, 166, 182, or 196.

In a further embodiment, the antibodies, antibody variants or antibody fragments of the invention comprise a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NOs: 13, 29, 45, 61, 65, 79, 95, 117, 131, 145, 151, 165, 181, or 195, and a light chain

variable region comprising the amino acid sequence of any one of SEQ ID NOs: 14, 30, 46, 62, 80, 96, 103, 118, 132, 146, 166, 182, or 196.

In yet another embodiment, the antibodies, antibody variants or antibody fragments of the invention neutralize dengue virus infection and comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 46; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 61 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 65 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 79 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 96; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 103; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 117 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 118; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 131 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 132; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 151 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 165 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 166; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 182; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 195 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 196.

Methods for chain replacement and for CDR grafting are well known in the art. Originally these methods were developed to humanize non-human antibodies (generally mouse antibodies) or to select human antibody counterparts having equivalent bioactivity to the non-

human antibodies. These methods include replacement techniques where only one of the CDRs, for example, the CDR3s, of the non-human antibody are retained and the remainder of the V-region, including the framework and the other two CDRs, for example, the CDRs 1 and 2, are individually replaced in steps performed sequentially (e.g. U.S. Patent Application No.

5 20030166871; Rader, et al., *Proc Natl Acad Sci USA* 95:8910-15, 1998; Steinberg, et al., *J Biol Chem* 275:36073-78, 2000; Rader, et al., *J Biol Chem* 275:13668-76, 2000).

In addition, methods of creating antibodies with the binding specificities of a reference antibody for a target antigen are described in Patent Application No. WO05/069,970. The methods include transferring, from the reference antibody to a recipient antibody or antibody  
10 fragment, the minimal essential binding specificity of the reference antibody. Examples of regions that can be transferred include, but are not limited to, the transfer of a single CDR segment, for example a CDR3 segment, from the heavy and/or from the light chain, or a D segment, or a CDR3-FR4 segment, or any CDR3-FR4 segment that comprises the minimal essential binding specificity determinant. Antibodies created using these methods retain the  
15 binding specificity, and often affinity, of the reference antibody.

The antibodies, antibody variants or antibody fragments of the invention include antibodies that comprise, *inter alia*, one or more CDRs, a heavy chain or a light chain of an exemplary antibody of the invention and retain their specificity and ability to neutralize dengue virus infection.

20 Exemplary antibodies of the invention include, but are not limited to, HMB-DV1, HMB-DV2, HMB-DV3, HMB-DV4, HMB-DV5, HMB-DV6, HMB-DV7, HMB-DV8, HMB-DV9, HMB-DV10, HMB-DV11, HMB-DV12, HMB-DV13, and HMB-DV14.

Variants of HMB-DV4 consist of a heavy chain variants having amino acid sequence recited in SEQ ID NO: 61 and SEQ ID NO: 65, and a light chain having the amino acid sequence  
25 recited in SEQ ID NO: 62. The nucleic acid sequences encoding the heavy chain variants are recited in SEQ ID NO: 63 and SEQ ID NO: 66. The nucleic acid encoding the light chain is recited in SEQ ID NO: 64. Thus, antibodies comprising the HMB-DV4 variant heavy chains (SEQ ID NOs: 61, 65) and light chain (SEQ ID NO: 62) are included within the scope of the invention.

30 As used herein, the term "HMB-DV4" is used to refer to any and/or all variants of HMB-DV4, for example, those with heavy chains corresponding to SEQ ID NOs: 61 and 65 and light chain corresponding to SEQ ID NO: 62.

In one embodiment, an antibody cocktail of the invention comprises two or more antibodies selected from the group consisting of HMB-DV1, HMB-DV2, HMB-DV3, HMB-DV4, HMB-DV5, HMB-DV6, HMB-DV7, HMB-DV8, HMB-DV9, HMB-DV10, HMB-DV11, HMB-DV12, HMB-DV13, and HMB-DV14. In another embodiment, a cocktail of the invention  
5 comprises three antibodies selected from the group consisting of HMB-DV1, HMB-DV2, HMB-DV3, HMB-DV4, HMB-DV5, HMB-DV6, HMB-DV7, HMB-DV8, HMB-DV9, HMB-DV10, HMB-DV11, HMB-DV12, HMB-DV13, and HMB-DV14. In yet another embodiment, an antibody cocktail of the invention comprises more than three, for example, 4, 5, 6, 7, or 8 antibodies selected from the group consisting of HMB-DV1, HMB-DV2, HMB-DV3, HMB-DV4, HMB-DV5, HMB-DV6, HMB-DV7, HMB-DV8, HMB-DV9, HMB-DV10, HMB-DV11, HMB-DV12, HMB-DV13, and HMB-DV14. In an exemplary embodiment, a cocktail of the  
10 invention comprises HMB-DV5, HMB-DV6, and HMB-DV8.

The invention further comprises an antibody, or fragment thereof, that binds to an epitope capable of binding to an antibody of the invention. The invention also comprises an antibody or  
15 an antibody fragment that competes with an antibody of the invention.

In another aspect, the invention also includes nucleic acid sequences encoding part or all of the light and heavy chains and CDRs of the antibodies of the present invention. In one embodiment, nucleic acid sequences according to the invention include nucleic acid sequences having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least  
20 98%, or at least 99% identity to the nucleic acid encoding a heavy or light chain of an antibody of the invention. In another embodiment, a nucleic acid sequence of the invention has the sequence of a nucleic acid encoding a heavy or light chain CDR of an antibody of the invention. For example, a nucleic acid sequence according to the invention comprises a sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at  
25 least 99% identical to the nucleic acid sequences of SEQ ID NOs: 7-12, 23-28, 39-44, 55-60, 73-78, 89-94, 101, 102, 111-116, 126-128, 129, 130, 140-144, 150, 159-164, 175-180, and 190-194.

Due to the redundancy of the genetic code, variants of these sequences will exist that encode the same amino acid sequences. These variants are included within the scope of the invention.

30 Variant antibodies are also included within the scope of the invention. Thus, variants of the sequences recited in the application are also included within the scope of the invention. Such variants include natural variants generated by somatic mutation *in vivo* during the immune response or *in vitro* upon culture of immortalized B cell clones. Alternatively, variants may arise

due to the degeneracy of the genetic code, as mentioned above or may be produced due to errors in transcription or translation. Variants may also be introduced to modify the antibody effector function, for instance in the Fc region to reduce the binding of the antibody to an Fc receptor.

Further variants of the antibody sequences having improved affinity and/or potency may be obtained using methods known in the art and are included within the scope of the invention. For example, amino acid substitutions may be used to obtain antibodies with further improved affinity. Alternatively, codon optimisation of the nucleotide sequence may be used to improve the efficiency of translation in expression systems for the production of the antibody. Further, polynucleotides comprising a sequence optimized for antibody specificity or neutralizing activity by the application of a directed evolution method to any of the nucleic acid sequences of the invention are also within the scope of the invention.

In one embodiment variant antibody sequences may share 70% or more (*i.e.* 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more) amino acid sequence identity with the sequences recited in the application. In some embodiments such sequence identity is calculated with regard to the full length of the reference sequence (*i.e.* the sequence recited in the application). In some further embodiments, percentage identity, as referred to herein, is as determined using BLAST version 2.1.3 using the default parameters specified by the NCBI (the National Center for Biotechnology Information).

Further included within the scope of the invention are vectors, for example expression vectors, comprising a nucleic acid sequence according to the invention. Cells transformed with such vectors are also included within the scope of the invention. Examples of such cells include but are not limited to, eukaryotic cells, *e.g.* yeast cells, animal cells or plant cells. In one embodiment the cells are mammalian, *e.g.* human, CHO, HEK293T, PER.C6, NS0, myeloma or hybridoma cells.

The invention also relates to monoclonal antibodies that bind to an epitope capable of binding an antibody of the invention. In one embodiment, the invention includes a monoclonal antibody that binds to an epitope capable of binding a monoclonal antibody selected from the group consisting of HMB-DV1, HMB-DV2, HMB-DV3, HMB-DV4, HMB-DV5, HMB-DV6, HMB-DV7, HMB-DV8, HMB-DV9, HMB-DV10, HMB-DV11, HMB-DV12, HMB-DV13, and HMB-DV14.

Monoclonal and recombinant antibodies are particularly useful in identification and purification of the individual polypeptides or other antigens against which they are directed. The

antibodies of the invention have additional utility in that they may be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA). In these applications, the antibodies can be labelled with an analytically-detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme. The antibodies may also be used for the  
5 molecular identification and characterisation (epitope mapping) of antigens.

Antibodies of the invention will typically be glycosylated. N-linked glycans attached to the C<sub>H</sub>2 domain of a heavy chain, for instance, can influence C1q and FcR binding, with aglycosylated antibodies having lower affinity for these receptors. The glycan structure can also affect activity *e.g.* differences in complement-mediated cell death may be seen depending on the  
10 number of galactose sugars (0, 1 or 2) at the terminus of a glycan's biantennary chain. An antibody's glycans preferably do not lead to a human immunogenic response after administration.

Antibodies of the invention can be coupled to a drug for delivery to a treatment site or coupled to a detectable label to facilitate imaging of a site comprising cells of interest, such as  
15 cells infected with dengue virus. Methods for coupling antibodies to drugs and detectable labels are well known in the art, as are methods for imaging using detectable labels. Labelled antibodies may be employed in a wide variety of assays, employing a wide variety of labels. Detection of the formation of an antibody-antigen complex between an antibody of the invention and an epitope of interest (a DENV epitope) can be facilitated by attaching a detectable  
20 substance to the antibody. Suitable detection means include the use of labels such as radionuclides, enzymes, coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, prosthetic group complexes, free radicals, particles, dyes, and the like. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group  
25 complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material is luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include <sup>125</sup>I, <sup>131</sup>I, <sup>35</sup>S, or <sup>3</sup>H.  
30 Such labeled reagents may be used in a variety of well-known assays, such as radioimmunoassays, enzyme immunoassays, *e.g.*, ELISA, fluorescent immunoassays, and the like.

An antibody according to the invention may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive metal ion or radioisotope. Examples of radioisotopes include, but are not limited to, I-131, I-123, I-125, Y-90, Re-188, Re-186, At-211, Cu-67, Bi-212, Bi-213, Pd-109, Tc-99, In-111, and the like. Such antibody conjugates can be used for modifying a given biological response; the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, calicheamicin bacterial toxin, or diphtheria toxin.

Techniques for conjugating such therapeutic moiety to antibodies are well known. See, for example, Arnon *et al.* (1985) "Monoclonal Antibodies for Immunotargeting of Drugs in Cancer Therapy," in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld *et al.* (Alan R. Liss, Inc.), pp. 243-256; ed. Hellstrom *et al.* (1987) "Antibodies for Drug Delivery," in *Controlled Drug Delivery*, ed. Robinson *et al.* (2d ed; Marcel Dekker, Inc.), pp. 623-653; Thorpe (1985) "Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological and Clinical Applications*, ed. Pinchera *et al.* pp. 475-506 (Editrice Kurtis, Milano, Italy, 1985); "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy," in *Monoclonal Antibodies for Cancer Detection and Therapy*, ed. Baldwin *et al.* (Academic Press, New York, 1985), pp. 303-316; and Thorpe *et al.* (1982) *Immunol. Rev.* 62:119-158.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described in reference 4. In addition, linkers may be used between the labels and the antibodies of the invention [5]. Antibodies or, antigen-binding fragments thereof may be directly labelled with radioactive iodine, indium, yttrium, or other radioactive particle known in the art [6]. Treatment may consist of a combination of treatment with conjugated and non-conjugated antibodies administered simultaneously or subsequently [7, 8].

Antibodies of the invention may also be attached to a solid support.

Additionally, antibodies of the invention, or functional antibody fragments thereof, can be chemically modified by covalent conjugation to a polymer to, for example, increase their circulating half-life, for example. Examples of polymers, and methods to attach them to peptides, are shown in references 9-12. In some embodiments the polymers may be selected from polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula:  $R(O-CH_2-CH_2)_nO-R$  where R can be hydrogen, or a

protective group such as an alkyl or alkanol group. In one embodiment the protective group may have between 1 and 8 carbons. In a further embodiment the protective group is methyl. The symbol  $n$  is a positive integer. In one embodiment  $n$  is between 1 and 1,000. In another embodiment  $n$  is between 2 and 500. In one embodiment the PEG has an average molecular weight between 1,000 and 40,000. In a further embodiment the PEG has a molecular weight between 2,000 and 20,000. In yet a further embodiment the PEG has a molecular weight of between 3,000 and 12,000. In one embodiment PEG has at least one hydroxy group. In another embodiment the PEG has a terminal hydroxy group. In yet another embodiment it is the terminal hydroxy group which is activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/antibody of the present invention.

Antibodies of the invention can be modified by introducing random amino acid mutations into particular region of the CH2 or CH3 domain of the heavy chain in order to alter their binding affinity for FcRn and/or their serum half-life in comparison to the unmodified antibodies. Examples of such modifications include, but are not limited to, substitutions of at least one amino acid from the heavy chain constant region selected from the group consisting of amino acid residues 250, 314, and 428.

Water-soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), and the like. In one embodiment, POG is used. Without being bound by any theory, because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, triglycerides, this branching would not necessarily be seen as a foreign agent in the body. In some embodiments POG has a molecular weight in the same range as PEG. The structure for POG is shown in reference 13, and a discussion of POG/IL-2 conjugates is found in reference 9.

Another drug delivery system that can be used for increasing circulatory half-life is the liposome. Methods of preparing liposome delivery systems are discussed in references 14, 15 and 16. Other drug delivery systems are known in the art and are described in, for example, references 17 and 18.

Antibodies of the invention may be provided in purified form. Typically, the antibody will be present in a composition that is substantially free of other polypeptides *e.g.* where less than 90% (by weight), usually less than 60% and more usually less than 50% of the composition is made up of other polypeptides.

Antibodies of the invention may be immunogenic in non-human (or heterologous) hosts *e.g.* in mice. In particular, the antibodies may have an idiotope that is immunogenic in non-human hosts, but not in a human host. Antibodies of the invention for human use include those that cannot be easily isolated from hosts such as mice, goats, rabbits, rats, non-primate  
5 mammals, *etc.* and cannot generally be obtained by humanisation or from xeno-mice.

Antibodies of the invention can be of any isotype (*e.g.* IgA, IgG, IgM *i.e.* an  $\alpha$ ,  $\gamma$  or  $\mu$  heavy chain), but will generally be IgG. Within the IgG isotype, antibodies may be IgG1, IgG2, IgG3 or IgG4 subclass. In one embodiment, the antibody is IgG1. Antibodies of the invention may have a  $\kappa$  or a  $\lambda$  light chain.

10 Included within the scope of the invention are DENV-neutralizing recombinant or engineered bispecific antibody molecules or antigen binding fragments thereof. Such antibodies and fragments may comprise a first binding site for an epitope on a first Dengue virus serotype and a second binding site for a second epitope on the same dengue virus serotype or on a different, for example, a second, third or fourth, dengue virus serotype. The variable domains of  
15 the respective binding sites can be formed as immunoglobulin isotypes of the invention or as heterodimeric Fab, Fab', F(ab')<sub>2</sub>, ScFv or diabodies that can be linked together via one or more peptide linkers.

### ***Production of antibodies***

Monoclonal antibodies according to the invention can be made by any method known in  
20 the art. The general methodology for making monoclonal antibodies using hybridoma technology is well known [19, 20]. Preferably, the alternative EBV immortalisation method described in reference 21 is used.

Using the method described in reference 21, B cells producing the antibody of the invention can be transformed with EBV in the presence of a polyclonal B cell activator.  
25 Transformation with EBV is a standard technique and can easily be adapted to include polyclonal B cell activators.

Additional stimulants of cellular growth and differentiation may optionally be added during the transformation step to further enhance the efficiency. These stimulants may be cytokines such as IL-2 and IL-15. In one aspect, IL-2 is added during the immortalisation step to  
30 further improve the efficiency of immortalisation, but its use is not essential.

The immortalised B cells produced using these methods can then be cultured using methods known in the art and antibodies isolated therefrom.

The antibodies of the invention can also be made by culturing single plasma cells in microwell culture plates using the method described in UK Patent Application 0819376.5. Further, from single plasma cell cultures, RNA can be extracted and single cell PCR can be performed using methods known in the art. The VH and VL regions of the antibodies can be amplified by RT-PCR, sequenced and cloned into an expression vector that is then transfected into HEK293T cells or other host cells. The cloning of nucleic acid in expression vectors, the transfection of host cells, the culture of the transfected host cells and the isolation of the produced antibody can be done using any methods known to one of skill in the art.

Monoclonal antibodies may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as HPLC or affinity chromatography. Techniques for purification of monoclonal antibodies, including techniques for producing pharmaceutical-grade antibodies, are well known in the art.

Fragments of the monoclonal antibodies of the invention can be obtained from the monoclonal antibodies by methods that include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, fragments of the monoclonal antibodies can be obtained by cloning and expression of part of the sequences of the heavy or light chains. Antibody "fragments" may include Fab, Fab', F(ab')<sub>2</sub> and Fv fragments. The invention also encompasses single-chain Fv fragments (scFv) derived from the heavy and light chains of a monoclonal antibody of the invention *e.g.* the invention includes a scFv comprising the CDRs from an antibody of the invention. Also included are heavy or light chain monomers and dimers as well as single chain antibodies, *e.g.* single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibodies or fragments or variants of the antibodies of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecules of the present invention or fragments thereof. Bacterial, for example *E. coli*, and other microbial systems may be used, in part, for expression of antibody fragments such as Fab and F(ab')<sub>2</sub> fragments, and especially Fv fragments and single chain antibody fragments, for example, single chain Fvs. Eukaryotic, *e.g.* mammalian, host cell expression systems may be used for production of larger antibody molecules, including complete

antibody molecules. Suitable mammalian host cells include CHO, HEK293T, PER.C6, NS0, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody of the invention comprising culturing a host cell comprising a vector of the present invention under  
5 conditions suitable for leading to expression of protein from DNA encoding the antibody of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line  
10 may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

Alternatively, antibodies according to the invention may be produced by i) expressing a nucleic acid sequence according to the invention in a cell, and ii) isolating the expressed  
15 antibody product. Additionally, the method may include iii) purifying the antibody.

### ***Screening and isolation of B cells***

Transformed B cells may be screened for those producing antibodies of the desired antigen specificity, and individual B cell clones may then be produced from the positive cells.

The screening step may be carried out by ELISA, by staining of tissues or cells (including  
20 infected or transfected cells), a neutralisation assay or one of a number of other methods known in the art for identifying desired antigen specificity. The assay may select on the basis of simple antigen recognition, or may select on the additional basis of a desired function *e.g.* to select neutralizing antibodies rather than just antigen-binding antibodies, to select antibodies that can change characteristics of targeted cells, such as their signalling cascades, their shape, their  
25 growth rate, their capability of influencing other cells, their response to the influence by other cells or by other reagents or by a change in conditions, their differentiation status, *etc.*

The cloning step for separating individual clones from the mixture of positive cells may be carried out using limiting dilution, micromanipulation, single cell deposition by cell sorting or another method known in the art.

30 The immortalised B cell clones of the invention can be used in various ways *e.g.* as a source of monoclonal antibodies, as a source of nucleic acid (DNA or mRNA) encoding a monoclonal antibody of interest, for research, *etc.*

The invention provides a composition comprising immortalised B memory cells, wherein the cells produce antibodies that neutralize one or more dengue virus serotypes, and wherein the antibodies are produced at  $\geq 5$ pg per cell per day. The invention also provides a composition comprising clones of an immortalised B memory cell, wherein the clones produce a monoclonal antibody that neutralizes one or more dengue virus serotypes, and wherein the antibody is produced at  $\geq 5$ pg per cell per day.

Exemplary immortalised B cell clone according to the invention include, but are not limited to, HMB-DV1, HMB-DV2, HMB-DV3, HMB-DV4, HMB-DV5, HMB-DV6, HMB-DV7, HMB-DV8, HMB-DV9, HMB-DV10, HMB-DV11, HMB-DV12, HMB-DV13, and HMB-DV14.

### ***Epitopes***

As mentioned above, the antibodies of the invention can be used to map the epitopes to which they bind. The epitopes recognised by the antibodies of the present invention may have a number of uses. The epitope and mimotopes thereof in purified or synthetic form can be used to raise immune responses (*i.e.* as a vaccine, or for the production of antibodies for other uses) or for screening patient serum for antibodies that immunoreact with the epitope or mimotopes thereof. In one embodiment such an epitope or mimotope, or antigen comprising such an epitope or mimotope may be used as a vaccine for raising an immune response. The antibodies and antigen binding fragments of the invention can also be used in a method of monitoring the quality of vaccines. In particular the antibodies can be used to check that the antigen in a vaccine contains the specific epitope in the correct conformation.

The epitope may also be useful in screening for ligands that bind to said epitope. Such ligands, include but are not limited to antibodies, including those from camels, sharks and other species, fragments of antibodies, peptides, phage display technology products, aptamers, adnectins, synthetic compounds, or fragments of other viral or cellular proteins, that may block the epitope and so prevent infection. Such ligands are encompassed within the scope of the invention.

### ***Recombinant expression***

The immortalised B memory cells of the invention may also be used as a source of nucleic acid for the cloning of antibody genes for subsequent recombinant expression. Expression from recombinant sources is more common for pharmaceutical purposes than

expression from B cells or hybridomas *e.g.* for reasons of stability, reproducibility, culture ease, *etc.*

Thus the invention provides a method for preparing a recombinant cell, comprising the steps of: (i) obtaining one or more nucleic acids (*e.g.* heavy and/or light chain genes) from the B  
5 cell clone that encodes the antibody of interest; and (ii) inserting the nucleic acid into an expression host in order to permit expression of the antibody of interest in that host.

Similarly, the invention provides a method for preparing a recombinant cell, comprising the steps of: (i) sequencing nucleic acid(s) from the B cell clone that encodes the antibody of interest; and (ii) using the sequence information from step (i) to prepare nucleic acid(s) for  
10 insertion into an expression host in order to permit expression of the antibody of interest in that host. The nucleic acid may, but need not, be manipulated between steps (i) and (ii) to introduce restriction sites, to change codon usage, to optimise transcription and/or translation regulatory sequences, and/or to modify effector function.

The invention also provides a method of preparing a recombinant cell, comprising the  
15 step of transforming a host cell with one or more nucleic acids that encode a monoclonal antibody of interest, wherein the nucleic acids are nucleic acids that were derived from an immortalised B cell clone of the invention. Thus the procedures for first preparing the nucleic acid(s) and then using it to transform a host cell can be performed at different times by different people in different places (*e.g.*, in different countries).

20 These recombinant cells of the invention can then be used for expression and culture purposes. They are particularly useful for expression of antibodies for large-scale pharmaceutical production. They can also be used as the active ingredient of a pharmaceutical composition. Any suitable culture techniques can be used, including but not limited to static culture, roller bottle culture, ascites fluid, hollow-fiber type bioreactor cartridge, modular  
25 minifermenter, stirred tank, microcarrier culture, ceramic core perfusion, *etc.*

Methods for obtaining and sequencing immunoglobulin genes from B cells are well known in the art (*e.g.*, see reference 22).

The expression host is preferably a eukaryotic cell, including yeast and animal cells, particularly mammalian cells (*e.g.* CHO cells, NS0 cells, human cells such as PER.C6 [Crucell;  
30 reference 23] or HKB-11 [Bayer; references 24 & 25] cells, myeloma cells [26 & 27], *etc.*), as well as plant cells. Preferred expression hosts can glycosylate the antibody of the invention, particularly with carbohydrate structures that are not themselves immunogenic in humans. In one embodiment the expression host may be able to grow in serum-free media. In a further

embodiment the expression host may be able to grow in culture without the presence of animal-derived products.

The expression host may be cultured to give a cell line.

The invention provides a method for preparing one or more nucleic acid molecules (*e.g.* heavy and light chain genes) that encode an antibody of interest, comprising the steps of:

5 (i) preparing an immortalised B cell clone according to the invention; (ii) obtaining from the B cell clone nucleic acid that encodes the antibody of interest. The invention also provides a method for obtaining a nucleic acid sequence that encodes an antibody of interest, comprising the steps of: (i) preparing an immortalised B cell clone according to the invention; (ii)

10 sequencing nucleic acid from the B cell clone that encodes the antibody of interest.

The invention also provides a method of preparing nucleic acid molecule(s) that encodes an antibody of interest, comprising the step of obtaining the nucleic acid from a B cell clone that was obtained from a transformed B cell of the invention. Thus the procedures for first obtaining the B cell clone and then preparing nucleic acid(s) from it can be performed at very different

15 times by different people in different places (*e.g.* in different countries).

The invention provides a method for preparing an antibody (*e.g.* for pharmaceutical use), comprising the steps of: (i) obtaining and/or sequencing one or more nucleic acids (*e.g.* heavy and light chain genes); (ii) using the sequence information from step (i) to prepare nucleic acid(s) for insertion into an expression host in order to permit expression of the antibody of interest in

20 that host; (iii) culturing or sub-culturing the expression host under conditions where the antibody of interest is expressed; and, optionally, (iv) purifying the antibody of the interest. The nucleic acid can, but need not be, obtained and/or sequenced from a B cell clone expressing the antibody of interest. In one embodiment, the nucleic acid from step (i) may, optionally be modified so as to introduce desired substitutions in the amino acid sequence of the antibody.

25 The invention also provides a method of preparing an antibody comprising the steps of: culturing or sub-culturing an expression host cell population under conditions where the antibody of interest is expressed and, optionally, purifying the antibody of the interest, wherein said expression host cell population has been prepared by (i) providing nucleic acid(s) encoding an antibody of interest; (ii) inserting the nucleic acid(s) into an expression host that can express the

30 antibody of interest, and (iii) culturing or sub-culturing expression hosts comprising said inserted nucleic acids to produce said expression host cell population.

***Pharmaceutical compositions***

The invention provides a pharmaceutical composition containing the antibodies and/or antibody fragments of the invention and/or nucleic acid encoding such antibodies and/or immortalised B cells that express such antibodies and/or the epitopes recognised by the antibodies of the invention. A pharmaceutical composition may also contain a pharmaceutically acceptable carrier to allow administration. The carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Within the scope of the invention, forms of administration may include those forms suitable for parenteral administration, *e.g.* by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. In one embodiment the compositions are adapted for administration to human subjects.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intraperitoneal, intrathecal, intraventricular, transdermal, transcutaneous, topical, subcutaneous, intranasal, enteral, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the

therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. Dosage treatment may be a single dose schedule or a multiple dose schedule. Known antibody-based pharmaceuticals provide guidance relating to frequency of administration *e.g.* whether a pharmaceutical should be delivered daily, weekly, monthly, *etc.* Frequency and dosage may also depend on the severity of symptoms.

Compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution, or suspension, in liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition, like Synagis™ and Herceptin™, for reconstitution with sterile water containing a preservative). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a patient. For example, a lyophilised antibody can be provided in kit form with sterile water or a sterile buffer.

It will be appreciated that the active ingredient in the composition will be an antibody molecule, an antibody fragment or variants and derivatives thereof. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, 20th edition, ISBN: 0683306472.

Pharmaceutical compositions of the invention generally have a pH between 5.5 and 8.5, in some embodiments this may be between 6 and 8, and in further embodiments about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen free.

The composition may be isotonic with respect to humans. In one embodiment pharmaceutical compositions of the invention are supplied in hermetically-sealed containers.

Pharmaceutical compositions will include a therapeutically effective amount of one or more antibodies of the invention and/or a polypeptide comprising an epitope that binds an antibody of the invention *i.e.* an amount that is sufficient to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic effect. Therapeutic effects also include reduction in physical symptoms. The precise effective amount for any particular subject will depend upon their size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. The effective amount for a given situation is determined by routine experimentation and is within the judgment of a clinician. For purposes of the present invention, an effective dose will generally be from about 0.01mg/kg to about 50mg/kg, or about 0.05 mg/kg to about 10 mg/kg of the compositions of the present invention in the individual to which it is administered. Known antibody-based pharmaceuticals provide guidance in this respect *e.g.*, Herceptin™ is administered by intravenous infusion of a 21 mg/ml solution, with an initial loading dose of 4mg/kg body weight and a weekly maintenance dose of 2mg/kg body weight; Rituxan™ is administered weekly at 375mg/m<sup>2</sup>; *etc.*

In one embodiment pharmaceutical compositions can include more than one (*e.g.* 2, 3, 4, 5, 6, 7, 8, *etc.*) antibody of the invention. In another embodiment the composition comprises two or more (*e.g.* 2, 3, 4, 5, *etc.*) antibodies, wherein the first antibody is specific for a first DENV epitope, and the second antibody is specific for a second DENV epitope. In yet another embodiment, the pharmaceutical composition comprises three antibodies of the invention. In another embodiment, the composition comprises two or more (*e.g.* 2, 3, 4, 5, *etc.*) antibodies, that together neutralise more than one dengue virus serotype. In yet another embodiment, the two or more antibodies of the invention together neutralise all four dengue virus serotypes, DENV-1, DENV-2, DENV-3 and DENV-4. In a further embodiment two or more antibodies of the invention together neutralise all four dengue virus serotypes by binding at least two distinct epitopes on each dengue virus serotype.

Exemplary antibodies of the invention for use in a pharmaceutical composition that neutralize a dengue virus without contributing to antibody-dependent enhancement of dengue virus infection include, but are not limited to, HMB-DV1, HMB-DV2, HMB-DV3, HMB-DV4, HMB-DV5, HMB-DV6, HMB-DV7, HMB-DV8, HMB-DV9, HMB-DV10, HMB-DV11, HMB-DV12, HMB-DV13, and HMB-DV14.

In one embodiment, a pharmaceutical composition includes two exemplary antibodies of the invention, for example, HMB-DV3 and HMB-DV7; HMB-DV3 and HMB-DV9; HMB-DV3 and HMB-DV12; HMB-DV3 and HMB-DV14; HMB-DV6 and HMB-DV7; HMB-DV6 and HMB-DV8. In another embodiment, a pharmaceutical composition includes three exemplary  
5 antibodies of the invention, for example, HMB-DV2, HMB-DV3 and HMB-DV6; HMB-DV2, HMB-DV6 and HMB-DV8; HMB-DV2, HMB-DV8 and HMB-DV9; HMB-DV2, HMB-DV8 and HMB-DV12; HMB-DV2, HMB-DV8 and HMB-DV14; HMB-DV5, HMB-DV6 and HMB-DV8. Based on the teachings herein, one of skill in the art can determine other combinations of antibodies for use in a pharmaceutical composition.

10 In one embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV1 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV2 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In another embodiment, the invention provides a  
15 pharmaceutical composition comprising the antibody HMB-DV3 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV4 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In yet another embodiment, the invention provides a pharmaceutical composition comprising the  
20 antibody HMB-DV5 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV6 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV7 or an antigen binding fragment  
25 thereof, and a pharmaceutically acceptable diluent or carrier.

In yet another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV8 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV9 or an antigen binding fragment  
30 thereof, and a pharmaceutically acceptable diluent or carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV10 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In yet another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV11 or an antigen binding fragment thereof, and a pharmaceutically acceptable

diluent or carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV12 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV13 or an antigen binding  
5 fragment thereof, and a pharmaceutically acceptable diluent or carrier. In yet another embodiment, the invention provides a pharmaceutical composition comprising the antibody HMB-DV14 or an antigen binding fragment thereof, and a pharmaceutically acceptable diluent or carrier.

Antibodies of the invention may be administered (either combined or separately) with  
10 other therapeutics *e.g.* with chemotherapeutic compounds, with radiotherapy, *etc.* Preferred therapeutic compounds include anti-viral compounds. Such combination therapy provides an additive or synergistic improvement in therapeutic efficacy relative to the individual therapeutic agents when administered alone. The term “synergy” is used to describe a combined effect of two or more active agents that is greater than the sum of the individual effects of each respective  
15 active agent. Thus, where the combined effect of two or more agents results in “synergistic inhibition” of an activity or process, it is intended that the inhibition of the activity or process is greater than the sum of the inhibitory effects of each respective active agent. The term “synergistic therapeutic effect” refers to a therapeutic effect observed with a combination of two or more therapies wherein the therapeutic effect (as measured by any of a number of parameters)  
20 is greater than the sum of the individual therapeutic effects observed with the respective individual therapies.

In compositions of the invention that include antibodies of the invention, the antibodies may make up at least 50% by weight (*e.g.* 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more) of the total protein in the composition. The antibodies are thus in purified form.

25 The invention provides a method of preparing a pharmaceutical, comprising the steps of: (i) preparing an antibody of the invention; and (ii) admixing the purified antibody with one or more pharmaceutically-acceptable carriers.

The invention also provides a method of preparing a pharmaceutical, comprising the step of admixing an antibody with one or more pharmaceutically-acceptable carriers, wherein the  
30 antibody is a monoclonal antibody that was obtained from a transformed B cell of the invention. Thus the procedures for first obtaining the monoclonal antibody and then preparing the pharmaceutical can be performed at very different times by different people in different places (*e.g.* in different countries).

As an alternative to delivering antibodies for therapeutic purposes, it is possible to deliver nucleic acid (typically DNA) that encodes the monoclonal antibody (or active fragment thereof) of interest to a subject, such that the nucleic acid can be expressed in the subject *in situ* to provide a desired therapeutic effect. Suitable gene therapy and nucleic acid delivery vectors are  
5 known in the art.

Compositions of the invention may be immunogenic compositions, and in some embodiments may be vaccine compositions comprising an antigen comprising a DENV epitope. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection).

10 Compositions may include an antimicrobial, particularly if packaged in a multiple dose format. Compositions may comprise detergent *e.g.* a Tween<sup>TM</sup> (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%. Compositions may include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10±2mg/ml NaCl is typical.

Compositions may comprise a sugar alcohol (*e.g.* mannitol) or a disaccharide (*e.g.* sucrose or trehalose) *e.g.* at around 15-30mg/ml (*e.g.* 25 mg/ml), particularly if they are to be  
15 lyophilised or if they include material which has been reconstituted from lyophilised material. The pH of a composition for lyophilisation may be adjusted to around 6.1 prior to lyophilisation.

The compositions of the invention may also comprise one or more immunoregulatory agents. In one embodiment, one or more of the immunoregulatory agents include(s) an adjuvant.

## 20 ***Medical treatments and uses***

The antibodies, antigen binding fragments, derivatives and variants thereof, or the cocktails and pharmaceutical compositions of the invention can be used for the treatment of DENV infection, for the prevention of DENV infection or for the diagnosis of DENV infection.

Methods of diagnosis may include contacting an antibody or an antibody fragment with a  
25 sample. Such samples may be tissue samples taken from, for example, salivary glands, lung, liver, pancreas, kidney, ear, eye, placenta, alimentary tract, heart, ovaries, pituitary, adrenals, thyroid, brain or skin. The methods of diagnosis may also include the detection of an antigen/antibody complex.

The invention therefore provides (i) an antibody, an antibody fragment, or variants and  
30 derivatives thereof according to the invention, (ii) an immortalised B cell clone according to the invention, (iii) an epitope capable of binding an antibody of the invention or (iv) a ligand,

preferably an antibody, capable of binding an epitope that binds an antibody of the invention for use in therapy.

Also provided is a method of treating a subject comprising administering to that subject (i) an antibody, an antibody fragment, variants and derivatives thereof, or a pharmaceutical  
5 composition according to the invention, or, a ligand, preferably an antibody, capable of binding an epitope that binds an antibody of the invention.

The invention also provides the use of (i) an antibody, an antibody fragment, or variants and derivatives thereof according to the invention, (ii) an immortalised B cell clone according to the invention, (iii) an epitope capable of binding an antibody of the invention, or (iv) a ligand,  
10 preferably an antibody, that binds to an epitope capable of binding an antibody of the invention, in the manufacture of a medicament for the prevention or treatment of DENV infection.

The invention provides a pharmaceutical composition for use as a medicament for the prevention or treatment of DENV infection. It also provides the use of an antibody of the invention and/or a protein comprising an epitope to which such an antibody binds in the  
15 manufacture of a medicament for treatment of a patient and/or diagnosis in a patient. It also provides a method for treating a subject, *e.g.*, a human subject. The method comprises the step of administering to the subject a therapeutically effective dose of a composition of the invention. One way of checking efficacy of therapeutic treatment involves monitoring disease symptoms after administration of the composition of the invention. Treatment can be a single dose  
20 schedule or a multiple dose schedule.

In one embodiment, an antibody, antibody fragment, antibody variant, epitope or pharmaceutical composition according to the invention is administered to a subject in need of such treatment. Such a subject includes, but is not limited to, one who is particularly at risk of or susceptible to DENV infection.

25 Antibodies of the invention can be used in passive immunisation. Antibodies and fragments or variants thereof, or a nucleic acid encoding an antibody or an antibody fragment or variant as described in the present invention may also be used in a kit for the diagnosis of dengue virus infection.

Epitopes capable of binding an antibody of the invention, *e.g.*, the monoclonal antibodies  
30 HMB-DV1, HMB-DV2, HMB-DV3, HMB-DV4, HMB-DV5, HMB-DV6, HMB-DV7, HMB-DV8, HMB-DV9, HMB-DV10, HMB-DV11, HMB-DV12, HMB-DV13, and HMB-DV14, may be used in a kit for monitoring the efficacy of vaccination procedures by detecting the presence of protective anti-DENV antibodies.

Antibodies, antibody fragments, or variants and derivatives thereof, as described in the present invention may also be used in a kit for monitoring vaccine manufacture with the desired immunogenicity.

The invention also provides a method of preparing a pharmaceutical composition,  
5 comprising the step of admixing a monoclonal antibody with one or more pharmaceutically-acceptable carriers, wherein the monoclonal antibody is a monoclonal antibody that was obtained from an expression host of the invention. Thus the procedures for first obtaining the monoclonal antibody (*e.g.* expressing it and/or purifying it) and then admixing it with the pharmaceutical carrier(s) can be performed at very different times by different people in different places (*e.g.* in  
10 different countries).

Starting with a transformed B cell of the invention, various steps of culturing, sub-culturing, cloning, sub-cloning, sequencing, nucleic acid preparation *etc.* can be performed in order to perpetuate the antibody expressed by the transformed B cell, with optional optimisation at each step. In a preferred embodiment, the above methods further comprise  
15 techniques of optimisation (*e.g.* affinity maturation or optimisation) applied to the nucleic acids encoding the antibody. The invention encompasses all cells, nucleic acids, vectors, sequences, antibodies *etc.* used and prepared during such steps.

In all these methods, the nucleic acid used in the expression host may be manipulated to insert, delete or amend certain nucleic acid sequences. Changes from such manipulation include,  
20 but are not limited to, changes to introduce restriction sites, to amend codon usage, to add or optimise transcription and/or translation regulatory sequences, *etc.* It is also possible to change the nucleic acid to alter the encoded amino acids. For example, it may be useful to introduce one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) amino acid substitutions, deletions and/or insertions into the antibody's amino acid sequence. Such point mutations can modify effector functions,  
25 antigen-binding affinity, post-translational modifications, immunogenicity, *etc.*, can introduce amino acids for the attachment of covalent groups (*e.g.* labels) or can introduce tags (*e.g.* for purification purposes). Mutations can be introduced in specific sites or can be introduced at random, followed by selection (*e.g.* molecular evolution). For instance, one or more nucleic acids encoding any of the CDR regions, heavy chain variable regions or light chain variable  
30 regions of antibodies of the invention can be randomly or directionally mutated to introduce different properties in the encoded amino acids. Such changes can be the result of an iterative process wherein initial changes are retained and new changes at other nucleotide positions are introduced. Moreover, changes achieved in independent steps may be combined. Different

properties introduced into the encoded amino acids may include, but are not limited to, enhanced affinity.

### ***General***

The term “comprising” encompasses “including” as well as “consisting” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional *e.g.* X + Y.

The word “substantially” does not exclude “completely” *e.g.* a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

The term “about” in relation to a numerical value  $x$  means, for example,  $x \pm 10\%$ .

The term “disease” as used herein is intended to be generally synonymous, and is used interchangeably with, the terms “disorder” and “condition” (as in medical condition), in that all reflect an abnormal condition of the human or animal body or of one of its parts that impairs normal functioning, is typically manifested by distinguishing signs and symptoms, and causes the human or animal to have a reduced duration or quality of life.

As used herein, reference to “treatment” of a patient is intended to include prevention and prophylaxis as well as therapy. The term “patient” means all mammals including humans. Generally, the patient is a human.

### **EXAMPLES**

Exemplary embodiments of the present invention are provided in the following examples. The following examples are presented only by way of illustration and to assist one of ordinary skill in using the invention. The examples are not intended in any way to limit the scope of the invention.

#### ***Example 1. Cloning of B cells and screening for identification of Dengue virus specific Abs.***

Memory B cells were isolated from the blood of DENV immune donors and immortalized using EBV and CpG as described in reference. Briefly, IgG<sup>+</sup> memory B cells were isolated using CD22 beads, followed by removal of IgM<sup>+</sup>, IgD<sup>+</sup> IgA<sup>+</sup> B cells using specific antibodies and cell sorting. The sorted cells (IgG<sup>+</sup>) were immortalized with EBV in the presence of CpG 2006 and irradiated allogeneic mononuclear cells. Replicate cultures each containing 30-50 memory B cells were set up in several 96 well U-bottom plates. After two weeks the culture supernatants were collected and tested for their capacity to stain C6/36 cells infected with DENV

of serotypes 1, 2, 3 or 4 by immunofluorescence analysis and/or to bind to recombinant DENV1-4 E2 proteins by ELISA. Supernatants were tested for their capacity to neutralize DENV infection of either VERO cells or DC-SIGN-transfected Raji cells and to enhance infection of K562 cells. B cell clones were isolated from positive polyclonal cultures as described previously  
 5 [28]. IgG concentrations in the supernatants of selected clones were determined using an IgG-specific ELISA.

***Example 2. Human mAbs from immortalized B cells recognize Dengue virus proteins and neutralize infection.***

For the viral neutralization and viral enhancement assay, titrated amounts of attenuated  
 10 DENV of serotypes 1, 2, 3 or 4 were mixed with an equal volume of culture supernatants. Viruses and multiplicity of infection (MOI) used were: rDEN1Δ30 (03JB186-1A+V2) MOI 0.04; rDEN2/4Δ30 (04JBV351-1A-V2) MOI 0.04; rDEN3/4Δ30 (DEN3#107C) MOI 0.02; rDEN4Δ30 (06JBV591-V3+1A1+v2) MOI 0.04. After 1-hour incubation at room temperature the mixture was added to target cells (e.g. VERO cells, DC-SIGN-Raji cells or K562 cells) in 96  
 15 well flat bottom plates and incubated at 37°C for 72-96 hours. The cells were then stained with a mouse monoclonal antibody to Dengue virus 1-4 E proteins (clone 4G2), followed by a fluorescein-labeled goat anti mouse Ig and analyzed by FACS. The neutralizing titer is indicated as the concentration of antibody (μg/ml) that gives a 50% reduction of DENV infection.

For identification of the target antigens recognized by the monoclonal antibodies yeasts  
 20 displaying Dengue virus E protein domains III or domain I-II were stained with the monoclonal antibodies followed by Cy5-labeled goat anti human IgG antibodies and analyzed by FACS. Western blotting experiments were performed using lysates of DENV-infected cells.

Table 3 shows that three different types of antibodies have been identified. They include those that are specific for domain III (DIII) of E protein, those that are specific for domains I-II (DI-II) of E protein and those specific for prM. The antibodies show different degrees of cross-reactivity with the 4 different DENV serotypes and neutralize those serotypes to which they  
 25 bind.

Table 3. Target Antigen Specificity of Neutralizing anti-Dengue Virus Antibodies

| Antibody  | Target Antigen | Dengue Virus Serotypes Neutralized |
|-----------|----------------|------------------------------------|
| HMB-DV-1  | E, DIII        | 1,2,3                              |
| HMB-DV-2  | E, DIII        | 1,3                                |
| HMB-DV-3  | prM            | 1,2,3,4                            |
| HMB-DV-4  | E, DI-II       | 1,2,3,4                            |
| HMB-DV-5  | E, DI-II       | 1,2,3,4                            |
| HMB-DV-6  | E, DIII        | 1,2,3                              |
| HMB-DV-7  | E, DIII        | 1,2,3                              |
| HMB-DV- 8 | E              | 4                                  |
| HMB-DV- 9 | E, DIII        | 2                                  |
| HMB-DV-10 | E, DIII        | 1,2,3,4                            |
| HMB-DV-11 | E, DIII        | 1,2,3,4                            |
| HMB-DV-12 | E, DI-DII      | 2                                  |
| HMB-DV-13 | E, DIII        | 1,2,3,4                            |
| HMB-DV-14 | E, DIII        | 2                                  |

Table 4 shows the results of virus neutralization assays on VERO cells and DC-SIGN-transfected Raji cells.

Table 4. Neutralization of Dengue Virus (serotypes DENV1-DENV4) by Antibodies

| Antibody | Cell type    | Neutralization                  |       |       |       |
|----------|--------------|---------------------------------|-------|-------|-------|
|          |              | EC <sub>50</sub> values (µg/ml) |       |       |       |
|          |              | DENV1                           | DENV2 | DENV3 | DENV4 |
| HMB-DV-1 | VERO         | 0.013                           | 0.577 | 0.014 | > 20  |
|          | DC-SIGN-Raji | 0.032                           | 5.340 | 0.055 | > 20  |
| HMB-DV-2 | VERO         | 0.006                           | > 20  | 0.006 | > 20  |
|          | DC-SIGN-Raji | 0.014                           | > 20  | 0.013 | > 20  |
| HMB-DV-3 | VERO         | 0.912                           | 1.615 | 0.120 | 0.070 |
|          | DC-SIGN-Raji | ND                              | ND    | ND    | ND    |
| HMB-DV-4 | VERO         | 0.591                           | 0.251 | 0.809 | 0.367 |
|          | DC-SIGN-Raji | 2.250                           | 1.370 | 0.613 | > 20  |
| HMB-DV-5 | VERO         | 0.066                           | 0.034 | 0.118 | 0.200 |
|          | DC-SIGN-Raji | 2.390                           | 0.504 | 0.348 | > 20  |
| HMB-DV-6 | VERO         | 0.008                           | 0.002 | 0.011 | > 20  |
|          | DC-SIGN-Raji | 0.027                           | 0.440 | 0.332 | > 20  |
| HMB-DV-7 | VERO         | 0.016                           | 0.004 | 0.020 | > 20  |
|          | DC-SIGN-Raji | ND                              | ND    | ND    | ND    |
| HMB-DV-8 | VERO         | > 20                            | > 20  | > 20  | 0.006 |
|          | DC-SIGN-Raji | ND                              | ND    | ND    | ND    |

|                  |              |       |       |       |       |
|------------------|--------------|-------|-------|-------|-------|
| <b>HMB-DV-9</b>  | VERO         | > 20  | 0.002 | > 20  | > 20  |
|                  | DC-SIGN-Raji | ND    | ND    | ND    | ND    |
| <b>HMB-DV-10</b> | VERO         | > 20  | 0.084 | > 20  | 0.466 |
|                  | DC-SIGN-Raji | ND    | ND    | ND    | ND    |
| <b>HMB-DV-11</b> | VERO         | > 20  | 0.048 | > 20  | 0.520 |
|                  | DC-SIGN-Raji | ND    | ND    | ND    | ND    |
| <b>HMB-DV-12</b> | VERO         | ND    | 0.003 | ND    | ND    |
|                  | DC-SIGN-Raji | ND    | ND    | ND    | ND    |
| <b>HMB-DV-13</b> | VERO         | 0.993 | 3.326 | 1.513 | > 20  |
|                  | DC-SIGN-Raji | ND    | ND    | ND    | ND    |
| <b>HMB-DV-14</b> | VERO         | > 20  | 0.002 | > 20  | > 20  |
|                  | DC-SIGN-Raji | ND    | ND    | ND    | ND    |

ND: not determined

***Example 3. Neutralizing recombinant anti-Dengue virus antibodies with mutations in the Fc region do not cause enhancement of virus infection on K562 cells.***

Antibody-dependent enhancement (ADE) of dengue virus infection has been described in the literature. This property could limit the therapeutic effectiveness of anti-dengue virus antibodies for use in clinical situations. Therefore, mRNAs from the immortalized B cell lines expressing antibodies HMB-DV-5, HMB-DV-6 and HMB-DV-8 were isolated, cDNA was synthesized using oligo-dT specific primers, variable regions of heavy and light chain were sequenced and cloned into an expression vector using specific primers. Vectors were transfected into host cells for recombinant expression. In addition to recombinant production of the wild-type IgG1 antibodies, each of the heavy chains was mutated at amino acids 4 and 5 of CH2 domain by substituting an alanine in place of the natural leucine using site-directed mutagenesis thereby creating the LALA variant of each antibody. Both recombinant wild type and mutated antibodies were harvested from the expression cell lines and purified. Both wild-type IgG1 anti-dengue virus antibody and the LALA variant bound to the target protein in comparable manner (data not shown).

Virus neutralization and enhancement was determined as above on VERO cells and K562 cells. Each of the three antibodies has a defined molecular target as well as serotype target (see Table 3). Figure 1 shows that the unmodified recombinant antibodies neutralize target virus infection of VERO cells in a dose-dependent manner (DOTTED LINES). On K562 cells, a cell line that is not efficiently infected by Dengue viruses, the unmodified antibodies show an enhancement of viral infection at concentrations that are generally higher than those required for neutralization (SOLID LINES). The experiment was repeated using the LALA variants of each antibody. Figure 2 shows that each of the LALA variants of the recombinant anti-dengue virus antibodies also neutralized the target virus on VERO cells (DOTTED LINES) in a dose-dependent manner. However, each of the LALA antibodies did not show evidence of antibody-dependent enhancement of infection on K562

cells (SOLID LINES). Note, the dose-response is flat on the K562 cells at the concentrations of antibodies used in this experiment and the line appears very close to the X-axis.

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**THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

1. A pharmaceutical composition comprising one, two or three human antibodies, or antigen-binding fragments thereof and a pharmaceutically acceptable diluent or carrier, wherein said antibody, antibodies, or antigen-binding fragments thereof, bind to, and neutralize dengue virus, and wherein said antibody, antibodies or antigen-binding fragments thereof, comprise the heavy chain CDR1, CDR2 and CDR3 and the light chain CDR1, CDR2 and CDR3 sequences as set forth in: (i) SEQ ID NOs: 1-6, respectively; (ii) SEQ ID NOs: 17-22, respectively; (iii) SEQ ID NOs: 33-38, respectively; (iv) SEQ ID NOs: 49-54, respectively; (v) SEQ ID NOs: 67-72, respectively; (vi) SEQ ID NOs: 83-88, respectively; (vii) SEQ ID NOs: 83-85, 99, 53 and 100, respectively; (viii) SEQ ID NOs: 105-110, respectively; (ix) SEQ ID NOs: 121-123, 70, 124 and 125, respectively; (x) SEQ ID NOs: 135-137, 138, 109 and 139, respectively; (xi) SEQ ID NOs: 149, 136-138, 109 and 139, respectively; (xii) SEQ ID NOs: 153-158, respectively; (xiii) SEQ ID NOs: 169-174, respectively; or (xiv) SEQ ID NOs: 185-188, 37 and 189, respectively.
2. The pharmaceutical composition of claim 1, wherein the composition neutralizes dengue virus serotypes DENV-1, DENV-2, DENV-3, and DENV-4.
3. The pharmaceutical composition of claim 2, wherein the composition neutralizes dengue virus serotypes DENV-1, DENV-2, DENV-3, and DENV-4 by binding at least two distinct epitopes on each dengue virus serotype.
4. The pharmaceutical composition of any one of claims 1 to 3, wherein the Fc region of said antibodies, or antigen-binding fragments thereof, comprises a CH2 L4A mutation, a CH2 L5A mutation, or both.
5. The pharmaceutical composition of any one of claims 1 to 4, wherein said antibodies or said antigen-binding fragments thereof, neutralizes more than one dengue virus of two, three or four different dengue virus serotypes.

6. The pharmaceutical composition of any one of claims 1 to 5, wherein at least one of said antibodies, or antigen-binding fragments thereof, comprise: (i) a heavy chain variable region having at least 70% sequence identity to any one of SEQ ID NOs: 13, 29, 45, 61, 65, 79, 95, 117, 131, 145, 151, 165, 181, or 195; or (ii) a light chain variable region having at least 70% sequence identity to any one of SEQ ID NOs: 14, 30, 46, 62, 80, 96, 103, 118, 132, 146, 166, 182, or 196.

7. The pharmaceutical composition of any one of claims 1 to 6, wherein at least one of said antibodies, or antigen-binding fragments thereof, comprise: (i) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NOs: 13, 29, 45, 61, 65, 79, 95, 117, 131, 145, 151, 165, 181, or 195; or (ii) a light chain variable region comprising the amino acid sequence of any one of SEQ ID NOs: 14, 30, 46, 62, 80, 96, 103, 118, 132, 146, 166, 182, or 196.

8. The pharmaceutical composition of any one of claims 1 to 7, wherein at least one of said antibodies or antigen-binding fragments thereof, comprise: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 46; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 61 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 65 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 79 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 96; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 103; or a heavy chain variable region

comprising the amino acid sequence of SEQ ID NO: 117 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 118; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 131 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 132; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 151 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 165 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 166; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 182; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 195 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 196.

9. The pharmaceutical composition of any one of claims 1 to 8, comprising an antibody, antibodies or antigen-binding fragments thereof, comprising the heavy chain CDR1, CDR2 and CDR3 and the light chain CDRI, CDR2 and CDR3 sequences as set forth in: (v) SEQ ID NOs: 67-72, respectively; (vi) SEQ ID NOs: 83-88, respectively; or (viii) SEQ ID NOs: 105-110, respectively, or a combination thereof.

10. The pharmaceutical composition of any one of claims 1 to 9, wherein said pharmaceutical composition further comprises an agent for extending the half- life of the antibody or antigen binding fragment thereof.

11. A pharmaceutical composition comprising one or more nucleic acid molecules comprising a polynucleotide encoding an antibody, antibodies, or antigen-binding fragments thereof, binding to, and neutralizing dengue virus and a pharmaceutically acceptable diluent or carrier, wherein said antibody, antibodies or antigen-binding fragments thereof, comprise the heavy chain CDRI , CDR2 and CDR3 and the light chain CDR1, CDR2 and CDR3 sequences as set forth in: (i) SEQ ID NOs: 1-6, respectively; (ii) SEQ ID NOs: 17-22, respectively; (iii) SEQ

ID NOs: 33-38, respectively; (iv) SEQ ID NOs: 49-54, respectively; (v) SEQ ID NOs: 67-72, respectively; (vi) SEQ ID NOs: 83-88, respectively; (vii) SEQ ID NOs: 83-85, 99, 53 and 100, respectively; (viii) SEQ ID NOs: 105-110, respectively; (ix) SEQ ID NOs: 121-123, 70, 124 and 125, respectively; (x) SEQ ID NOs: 135-137, 138, 109 and 139, respectively; (xi) SEQ ID NOs: 149, 136-138, 109 and 139, respectively; (xii) SEQ ID NOs: 153-158, respectively; (xiii) SEQ ID NOs: 169-174, respectively; or (xiv) SEQ ID NOs: 185-188, 37 and 189, respectively.

12. A pharmaceutical composition comprising one or more nucleic acid molecules comprising a polynucleotide encoding an antibody, antibodies, or antigen-binding fragments thereof, binding to, and neutralizing dengue virus and a pharmaceutically acceptable diluent or carrier, wherein said antibody, antibodies or antigen-binding fragments thereof, comprise the heavy chain CDR1, CDR2 and CDR3 and the light chain CDR1, CDR2 and CDR3 sequences encoded by the sequences as set forth in: (i) SEQ ID NOs: 7-12, respectively; (ii) SEQ ID NOs: 23-28, respectively; (iii) SEQ ID NOs: 39-44, respectively; (iv) SEQ ID NOs: 55-60, respectively; (v) SEQ ID NOs: 73-78, respectively; (vi) SEQ ID NOs: 89-94, respectively; (vii) SEQ ID NOs: 89-91, 101, 59, 102, respectively; (viii) SEQ ID NOs: 111-116, respectively; (ix) SEQ ID NOs: 126-128, 76, 129, 130, respectively; (x) SEQ ID NOs: 140-143, 115, 144, respectively; (xi) SEQ ID NOs: 150, 141-143, 115, 144, respectively; (xii) SEQ ID NOs: 159-164, respectively; (xiii) SEQ ID NOs: 175-180, respectively; or (xiv) SEQ ID NOs: 190-193, 43, 194, respectively.

13. A cell expressing the antibody, antibodies, or an antigen-binding fragment thereof, of the pharmaceutical composition of any one of claims 1 to 9, or a cell expressing a vector comprising one or more nucleic acid molecules of the pharmaceutical composition of claim 11 or 12.

14. Use of a therapeutically or prophylactically effective amount of the pharmaceutical composition of any one of claims 1 to 12 to inhibit, prevent or treat a dengue virus infection.

15. Use of one, two or three human antibodies, or antigen-binding fragments thereof, or one or more nucleic acid molecules comprising a polynucleotide encoding said antibody, antibodies,

or antigen-binding fragments thereof, in the manufacture of a pharmaceutical composition to inhibit, prevent or treat a dengue virus infection; wherein said antibody, antibodies, or antigen-binding fragments thereof, bind to, and neutralize dengue virus, and wherein said antibody, antibodies, or antigen-binding fragments thereof comprise the heavy chain CDR1, CDR2 and CDR3 and the light chain CDR1, CDR2 and CDR3 sequences as set forth in: (i) SEQ ID NOs: 1-6, respectively; (ii) SEQ ID NOs: 17-22, respectively; (iii) SEQ ID NOs: 33-38, respectively; (iv) SEQ ID NOs: 49-54, respectively; (v) SEQ ID NOs: 67-72, respectively; (vi) SEQ ID NOs: 83-88, respectively; (vii) SEQ ID NOs: 83-85, 99, 53 and 100, respectively; (viii) SEQ ID NOs: 105-110, respectively; (ix) SEQ ID NOs: 121-123, 70, 124 and 125, respectively; (x) SEQ ID NOs: 135-137, 138, 109 and 139, respectively; (xi) SEQ ID NOs: 149, 136-138, 109 and 139, respectively; (xii) SEQ ID NOs: 153-158, respectively; (xiii) SEQ ID NOs: 169-174, respectively; or (xiv) SEQ ID NOs: 185-188, 37 and 189, respectively.

16. The use according to 15, additionally comprising use of a second therapeutic agent.

17. The use according to claim 16, wherein said second therapeutic agent is an anti-viral agent.

18. Use of the pharmaceutical composition of any one of claims 1 to 12, (i) in the manufacture of a medicament for the treatment of dengue virus infection, (ii) in a vaccine, or (iii) in diagnosis of dengue virus infection.

19. Use of the pharmaceutical composition of any one of claims 1 to 12, for monitoring the quality of anti-dengue virus vaccines by checking that the antigen of said vaccine contains the specific epitope in the correct conformation.

20. An isolated human antibody, or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof, bind to, and neutralize dengue virus, and wherein said antibody or antigen-binding fragment thereof, comprise the heavy chain CDR1, CDR2 and CDR3 and the light chain CDR1, CDR2 and CDR3 sequences as set forth in:

(i) SEQ ID NOs: 1-6, respectively;

- (ii) SEQ ID NOs: 17-22, respectively;
- (iii) SEQ ID NOs: 33-38, respectively;
- (iv) SEQ ID NOs: 49-54, respectively;
- (v) SEQ ID NOs: 67-72, respectively;
- (vi) SEQ ID NOs: 83-88, respectively;
- (vii) SEQ ID NOs: 83-85, 99, 53 and 100, respectively;
- (viii) SEQ ID NOs: 105-110, respectively;
- (ix) SEQ ID NOs: 121-123, 70, 124 and 125, respectively;
- (x) SEQ ID NOs: 135-137, 138, 109 and 139, respectively;
- (xi) SEQ ID NOs: 149, 136-138, 109 and 139, respectively;
- (xii) SEQ ID NOs: 153-158, respectively;
- (xiii) SEQ ID NOs: 169-174, respectively; or
- (xiv) SEQ ID NOs: 185-188, 37 and 189, respectively.

21. The antibody or antigen-binding fragment thereof of claim 20, wherein the antibody or antigen-binding fragment thereof neutralizes dengue virus serotypes DENV-1, DENV-2, DENV-3, and DENV-4.

22. The antibody or antigen-binding fragment thereof of claim 20, wherein the antibody or antigen-binding fragment thereof neutralizes dengue virus serotypes DENV-1, DENV-2, DENV-3, and DENV-4 by binding at least two distinct epitopes on each dengue virus serotype.

23. The antibody or antigen-binding fragment thereof of any one of claims 20 to 22, wherein the Fc region of said antibodies, or antigen-binding fragments thereof, comprises a CH2 L4A mutation, a CH2 L5A mutation, or both.

24. The antibody or antigen-binding fragment thereof of any one of claims 20 to 23, wherein said antibody or said antigen-binding fragment thereof, neutralizes more than one dengue virus of two, three or four different dengue virus serotypes.

25. The antibody or antigen-binding fragment thereof of any one of claims 20 to 24, wherein

said antibody or said antigen-binding fragment thereof, comprises:

- (i) a heavy chain variable region having at least 70% sequence identity to any one of SEQ ID NOs: 13, 29, 45, 61, 65, 79, 95, 117, 131, 145, 151, 165, 181, or 195; or
- (ii) a light chain variable region having at least 70% sequence identity to any one of SEQ ID NOs: 14, 30, 46, 62, 80, 96, 103, 118, 132, 146, 166, 182, or 196.

26. The antibody or antigen-binding fragment thereof of any one of claims 20 to 25, wherein said antibody or said antigen-binding fragment thereof comprises:

- (i) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NOs: 13, 29, 45, 61, 65, 79, 95, 117, 131, 145, 151, 165, 181, or 195; or
- (ii) a light chain variable region comprising the amino acid sequence of any one of SEQ ID NOs: 14, 30, 46, 62, 80, 96, 103, 118, 132, 146, 166, 182, or 196.

27. The antibody or antigen-binding fragment thereof of any one of claims 20 to 26, wherein said antibody or said antigen-binding fragment thereof: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 46; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 61 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 65 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 79 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 96; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 103; or a heavy chain variable region comprising the amino acid

sequence of SEQ ID NO: 117 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 118; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 131 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 132; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 151 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 165 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 166; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 182; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 195 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 196.

28. The antibody or antigen-binding fragment thereof of any one of claims 20 to 27, wherein said antibody or said antigen-binding fragment thereof comprises the heavy chain CDR1, CDR2 and CDR3 and the light chain CDR1, CDR2 and CDR3 sequences as set forth in: (v) SEQ ID NOs: 67-72, respectively; (vi) SEQ ID NOs: 83-88, respectively; or (viii) SEQ ID NOs: 105-110, respectively, or a combination thereof.

29. An isolated human antibody, or antigen-binding fragment thereof that neutralizes dengue virus serotypes DENV-1, DENV-2, DENV-3, or DENV-4, wherein the antibody or antigen-binding fragment does not contribute to antibody-dependent enhancement of dengue virus infection, wherein the Fc region of said antibodies, or antigen-binding fragments thereof, comprises a CH2 L4A mutation, a CH2 L5A mutation, or both, and said antibody or antigen-binding fragment thereof, comprises the heavy chain CDR1, CDR2 and CDR3 and the light chain CDR1, CDR2 and CDR3 sequences as set forth in:

- (i) SEQ ID NOs: 1-6, respectively;
- (ii) SEQ ID NOs: 17-22, respectively;
- (iii) SEQ ID NOs: 33-38, respectively;

- (iv) SEQ ID NOs: 49-54, respectively;
- (v) SEQ ID NOs: 67-72, respectively;
- (vi) SEQ ID NOs: 83-88, respectively;
- (vii) SEQ ID NOs: 83-85, 99, 53 and 100, respectively;
- (viii) SEQ ID NOs: 105-110, respectively;
- (ix) SEQ ID NOs: 121-123, 70, 124 and 125, respectively;
- (x) SEQ ID NOs: 135-137, 138, 109 and 139, respectively; (xi) SEQ ID NOs: 149, 136-138, 109 and 139, respectively;
- (xii) SEQ ID NOs: 153-158, respectively;
- (xiii) SEQ ID NOs: 169-174, respectively; or
- (xiv) SEQ ID NOs: 185-188, 37 and 189, respectively.

30. The antibody or antigen-binding fragment thereof of claim 29, wherein the antibody is a monoclonal antibody or a recombinant antibody.

31. The antibody or antigen-binding fragment thereof of claim 29 or 30, wherein said antibody or said antigen-binding fragment thereof, neutralizes more than one dengue virus of two, three or four different dengue virus serotypes.

32. The antibody or antigen-binding fragment of any one of claims 29 to 31, wherein the antibody or antigen-binding fragment comprises

- (i) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 13 and a light chain variable region having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 14; or
- (ii) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 29 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 30; or
- (iii) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 45 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 46; or
- (iv) a heavy chain variable region having at least 80% sequence identity to the amino acid

sequence of SEQ ID NOs: 61 or 65 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 62; or

(v) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 79 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 80; or

(vi) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 95 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 96; or

(vii) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 95 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 103; or

(viii) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 117 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 118; or (ix) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 131 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 132; or (x) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 145 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 146; or

(xi) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 151 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 146; or

(xii) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 165 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 166; or

(xiii) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 181 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 182; or

(xiv) a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 195 and a light chain variable region having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 196.

33. The antibody or antigen-binding fragment of any one of claims 29 to 31, wherein the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 46; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 61 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 65 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 79 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 96; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 95 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 103; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 117 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 118; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 131 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 132; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 151 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 146; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 165 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 166; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 182; or a heavy chain variable region comprising the

amino acid sequence of SEQ ID NO: 195 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 196.

34. The antibody or antigen-binding fragment of any one of claims 29 to 33, wherein the antibody is a monoclonal antibody or a single chain antibody, and the antigen-binding fragment is a Fab, Fab', F(ab')<sub>2</sub>, Fv or scFv.

Figure 1. Neutralization and antibody-dependent enhancement of wild-type mAbs

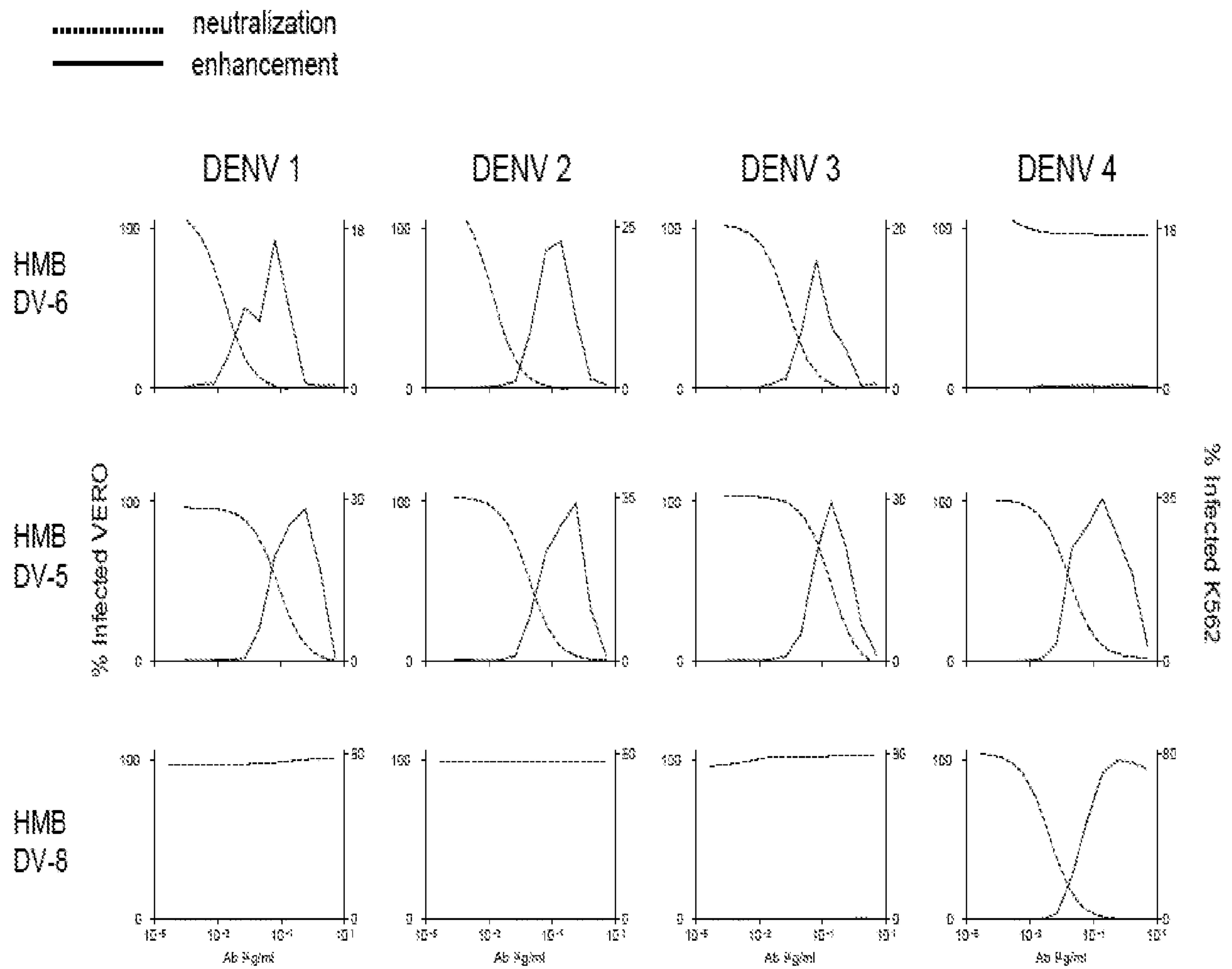


Figure 2. Neutralization and antibody-dependent enhancement of LALA-variant mAbs

