

Claims

1. Protein NS2b or at least one fragment thereof as biomarker or diagnostic marker for use in a method for the diagnosis and/or prognosis of Zika virus infections.
2. The NS2b protein or at least one fragment thereof for use according to claim 1, wherein the fragment comprises an amino acid sequence of the ZIKV polyproteome of positions 1429-1449 of Zika Uganda Strain MR766_NIID (SEQ ID NO. 1).
3. The NS2b protein or at least one fragment thereof for use according to claim 1 or 2, wherein the fragment is a peptide having a length of at least 5 amino acids, or at least 9 amino acids, such as 5 to 130 amino acids, or 5 to 100 amino acids, or 5 to 50 amino acids, or 9 to 50 amino acids, e.g. about 15 to 21 amino acids or about 9 to 15 amino acids or about 9 to 21 amino acids.
4. The NS2b protein or at least one fragment thereof for use according to any one of claims 1 to 3, wherein the at least one fragment comprises a peptide having
an amino acid sequence selected from SEQ ID Nos. 1 to 10 or combinations thereof,
or
an amino acid sequence having at least about 50 % sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60 %, more preferably more than about 70 or 80 or 90 % sequence identity.
5. The NS2b fragment for use according to any one of claims 1 to 4, wherein the fragment comprises or is a peptide having
an amino acid sequence selected from SEQ ID Nos. 1 to 10, or
an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1.

6. The NS2b fragment for use according to claim 5, wherein the amino acid substitution(s) are

A9P, A9E, A9M, A9S, A9T, A9K;

V11D, V11E, V11T, V11A, V11N, V11S, V11M, V11L or I11D, I11E, I11T, I11A, I11N, I11S, I11M, I11L;

S15D, S15K, S15M, S15A, S15R, S15N; and/or

R17D, R17E, R17T

preferably A9P, A9E, V11D, I11D, V11E, I11E, S15D, S15K, and/or R17D, R17E.

such as an amino acid sequence selected from SEQ ID Nos. 11 to 16.

7. The NS2b fragment for use according to any one of claims 1 to 6, wherein the fragment comprises or is a cyclic peptide having

an amino acid sequence selected from SEQ ID Nos. 1 to 10, or

an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

wherein the cyclization is preferably via

thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,

or

head-to-tail cyclization.

8. The NS2b protein or at least one fragment thereof for use according to any one of claims 1 to 7, wherein one fragment is used which comprises more than one of said peptides, or more than one fragment each comprising or consisting of one peptide are used.

9. The NS2b protein or at least one fragment thereof for use according to any one of claims 1 to 8, wherein said NS2b protein or said at least one fragment comprises at least one further component(s), which is/are preferably selected from

- label(s),

such as fluorescent label(s),

- tag(s),

preferably for immobilization, such as biotin, alkyn, azid, PEG,
 - linker or anchoring group(s)
 preferably for attachment of the label(s), and/or tag(s),
 such as cysteine, poly-GS-linker, e.g. GSGSG,
 or combinations thereof,

wherein, preferably, the at least one further component(s) is/are covalently coupled to said NS2b protein or said at least one fragment thereof via a linker, an amino acid side chain, and/or to the N- and/or C-terminus.

10. The NS2b protein or at least one fragment thereof for use according to any one of claims 1 to 9, wherein said method is an *in vitro* or *ex vivo* method.

11. The NS2b protein or at least one fragment thereof for use according to any one of the preceding claims, comprising the use in immunoassays, such as enzyme-linked immunosorbent assay (ELISA), bead-based immunoassays such as Luminex, plaque reduction neutralization test (PRNT), lateral flow or strip tests.

12. The NS2b protein or at least one fragment thereof for use according to any one of the preceding claims, comprising detecting an anti-Zika antibody response in a sample of a patient,

wherein said patient sample is preferably blood, more preferably selected from serum, plasma and whole blood.

13. A peptide comprising an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

wherein the amino acid substitution(s) are preferably

A9P, A9E, A9M, A9S, A9T, A9K;

V11D, V11E, V11T, V11A, V11N, V11S, V11M, V11L or I11D, I11E, I11T, I11A, I11N, I11S, I11M, I11L;

S15D, S15K, S15M, S15A, S15R, S15N; and/or

R17D, R17E, R17T,

more preferably A9P, A9E, V11D, I11D, V11E, I11E, S15D, S15K, and/or R17D, R17E.

such as an amino acid sequence selected from SEQ ID Nos. 11 to 16.

14. A cyclic peptide comprising
 an amino acid sequence selected from SEQ ID Nos. 1 to 10, or
 an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

wherein the cyclization is preferably via

thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,

or

head-to-tail cyclization.

15. The peptide of claim 13 or the cyclic peptide of claim 14 having a length of at least 5 amino acids or at least 9 amino acids,

such as 5 to 130 amino acids, or 5 to 100 amino acids, or 5 to 50 amino acids, or 9 to 50 amino acids, e.g. about 15 to 21 amino acids or about 9 to 15 amino acids or about 9 to 21 amino acids.

16. The peptide or the cyclic peptide of any one of claims 13 to 15, comprising at least one further component(s), which is/are preferably selected from

- label(s),

such as fluorescent label(s),

- tag(s),

preferably for immobilization, such as biotin, alkyn, azid, PEG,

- linker or anchoring group(s)

preferably for attachment of the label(s), and/or tag(s),

such as cysteine, poly-GS-linker, e.g. GSGSG,

or combinations thereof,

wherein said at least one further component(s) is/are covalently coupled to said peptide via a linker, an amino acid side chain, and/or to the N- and/or C-terminus.

17. A composition or an array comprising

(a) at least one peptide comprising

an amino acid sequence selected from SEQ ID NOs. 1 to 10, or

an amino acid sequence having at least about 50 % sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60 %, more preferably more than about 70 or 80 or 90 % sequence identity, or

(b) at least one peptide comprising

an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

wherein the amino acid substitution(s) are preferably A9P, A9E, A9M, A9S, A9T, A9K; V11D, V11E, V11T, V11A, V11N, V11S, V11M, V11L or I11D, I11E, I11T, I11A, I11N, I11S, I11M, I11L; S15D, S15K, S15M, S15A, S15R, S15N; and/or R17D, R17E, R17T, more preferably A9P, A9E, V11D, I11D, V11E, I11E, S15D, S15K, and/or R17D, R17E, such as an amino acid sequence selected from SEQ ID Nos. 11 to 16,

and/or

(c) at least one cyclic peptide according to claim 14 or 15.

18. A multimer compound comprising at least two of component(s) (a) to (d):

(a) a peptide comprising

an amino acid sequence selected from SEQ ID NOs. 1 to 10, or

an amino acid sequence having at least about 50 % sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60 %, more preferably more than about 70 or 80 or 90 % sequence identity, or

(b) a peptide of any one of claims 13, 15 or 16

(c) a cyclic peptide of any one of claims 14 to 16,

and/or

- (d) a NS2b fragment, as defined in any one of claims 1 to 9,

wherein at least two of component(s) (a) to (d) are covalently coupled to each other or are connected via a linear or cyclic scaffold.

19. The peptide or the cyclic peptide of any one of claims 13 to 16 or the composition or array of claim 17 or the multimer compound of claim 18 as biomarker or diagnostic marker for Zika virus infections.

20. The peptide or the cyclic peptide of any one of claims 13 to 16 or the composition or array of claim 17 or the multimer compound of claim 18 for use in a method of the diagnosis and/or prognosis of Zika virus infections, wherein said method is an *in vitro* or *ex vivo* method.

21. A peptide-based compound comprising

- (i) at least one peptide comprising
an amino acid sequence selected from SEQ ID NOs. 1 to 10, or
an amino acid sequence having at least about 50 % sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60 %, more preferably more than about 70 or 80 or 90 % sequence identity, or
an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

and/or at least one cyclic peptide according to claim 14,

and

- (ii) at least one further component, preferably covalently coupled to the peptide and/or cyclic peptide (i).

22. The peptide-based compound of claim 21, wherein the peptide and/or cyclic peptide has a length of at least 5 amino acids or at least 9 amino acids,

such as 5 to 130 amino acids, or 5 to 100 amino acids, or 5 to 50 amino acids, or 9 to 50 amino acids, e.g. about 15 to 21 amino acids or about 9 to 15 amino acids or about 9 to 21 amino acids.

23. The peptide-based compound of claim 21 or 22, wherein the at least one further component is selected from

- label(s), such as fluorescent label(s),
- tag(s), preferably for immobilization, such as biotin, alkyn, azid, PEG,
- linker or anchoring group(s) preferably for attachment of the label(s), and/or tag(s), such as cysteine, poly-GS-linker, e.g. GSGSG,
- or combinations thereof,

wherein the at least one further component(s) is/are preferably covalently coupled to said peptide and/or cyclic peptide via a linker, an amino acid side chain, and/or to the N- and/or C-terminus.

24. The peptide-based compound of any of claims 21 to 23 for use in a method of the diagnosis and/or prognosis of Zika virus infections, wherein said method is an *in vitro* or *ex vivo* method.

25. An *in vitro* method for the diagnosis and/or prognosis of Zika virus infections, comprising the steps of

- (a) providing a sample of a patient to be tested,
- (b) providing
 - (1) protein NS2b or at least one fragment thereof, as defined in any one of claims 1 to 9;
 - (2) at least one peptide-based compound of any of claims 21 to 23;
 - (3) at least one peptide of any of claims 13, 15 or 16;
 - (4) at least one cyclic peptide of any of claims 14 to 16;
 - (5) a composition or an array of claim 17;
 and/or
 - (6) a multimer compound of claim 18;
- (c) performing an immunoassay, comprising detecting an anti-Zika antibody response in said patient sample,

wherein said patient sample is preferably blood, more preferably selected from serum, plasma and whole blood.

26. The method of claim 25, comprising the use of a negative control, wherein said negative control is preferably a peptide, cyclic peptide or a peptide-based compound according to any one of claims 13 to 16 or 19 to 21 comprising an amino acid substitution in position K7 in reference to the amino acid sequence of SEQ ID NO. 1.