

CLAIMS

1. A modified dengue virus, comprising

a recoded envelope (E) protein, having a reduced codon pair bias compared to its parent E protein encoding sequence, or both the recoded envelope (E) protein encoding

5 sequence and a recoded prM protein encoding sequence having a reduced codon pair bias compared to its parent prM protein encoding sequence,

in which the recoded E protein encoding sequence is about a continuous one-third to one-half of the length of an entire E protein encoding sequence,

in which the codon pair bias of the recoded E protein-encoding sequence is reduced by

10 at least 0.05,

in which the parent E protein encoding sequence is selected from serotype 1 DENV-1/VN/BID-V1774/2007 (SEQ ID NO:1), serotype 2 DENV-2/NI/BID-V533/2005 (SEQ ID NO:3), serotype 3 DENV-3/VE/BID-V2268/2008 (SEQ ID NO:5), serotype 4 DENV-4/US/BID-V2448/1999 (SEQ ID NO:7) and combinations thereof.
- 15 2. A modified dengue virus according to Claim 1, in which expression of the E protein or both the E protein and the prM protein are reduced compared to its parent dengue virus.
3. A modified dengue virus according to Claim 1, in which the recoded E protein encoding sequence or both the recoded E-protein encoding sequence and the recoded prM-encoding sequence has a codon pair bias of less than -0.05.
- 20 4. A tetravalent dengue virus composition comprising: a modified dengue virus according to Claim 1 of serotype 1, a modified dengue virus according to Claim 1 of serotype 2, a modified dengue virus according to Claim 1 of serotype 3 and a modified dengue virus according to Claim 1 of serotype 4.
5. A dengue vaccine composition for inducing a protective immune response in a subject,
- 25 comprising a modified dengue virus according to Claim 1, 2 or 3, or a tetravalent dengue virus composition according to Claim 4, and a pharmaceutically acceptable excipient or carrier.
6. A modified dengue virus according to Claim 1, 2 or 3 or a tetravalent dengue virus composition according to Claim 4 for use in a method of eliciting an immune response in a

subject, in which an effective dose of a composition comprising the modified dengue virus is to be administered to a subject.

7. A modified dengue virus for use according to Claim 6 or a tetravalent dengue virus composition according to Claim 4, in which the immune response is a protective immune response, and a prophylactically effective or therapeutically effective dose of a dengue vaccine composition according to Claim 5 is to be administered to the subject.
8. A modified dengue virus for use according to Claim 6 or a tetravalent dengue virus composition according to Claim 4, in which at least one adjuvant is to be further administered to the subject.
9. A modified dengue virus for use according to Claim 6 or a tetravalent dengue virus composition according to Claim 4, in which the immune response is cross-protective against a heterologous dengue virus.
10. A combination of:
 - (i) an attenuated dengue virus produced by a method other than codon-pair deoptimization or codon deoptimization, or increasing of CpG or UpA di-nucleotides and
 - (ii) a modified dengue virus according to Claim 1, 2 or 3 or a composition according to Claim 4,
 for use in a method of eliciting an immune response in a subject in need thereof,

 in which a prime dose of (i) or (ii) is to be administered to the subject,

 in which one or more boost dose of (i) or (ii) is to be administered to the subject;

 in which at least the prime dose or the one or more boost dose is the modified dengue virus.
11. A combination for use according to Claim 10, in which a first of the one or more boost dose is to be administered about 2 weeks after the prime dose.
12. A combination for use according to Claim 10, in which expression of the E protein or prM protein or both are reduced compared to its parent dengue virus.

13. A combination for use according to Claim 10, in which the recoded E protein encoding sequence or both the recoded E-protein encoding sequence and the recoded prM-encoding sequence has a codon pair bias of less than -0.05.

14. A combination for use according to Claim 10, in which (ii) comprises a composition
5 according to Claim 4.

15. A method of making a modified dengue virus genome comprising:

obtaining a parent E protein encoding sequence of a parent dengue virus;

recoding the E protein encoding sequence to reduce protein expression, in which the recoded envelope (E) protein encoding sequence has a reduced codon pair bias

10 compared to its parent E protein encoding sequence, or both the recoded envelope (E) protein encoding sequence and a recoded prM protein encoding sequence having a reduced codon pair bias compared to its parent prM protein encoding sequence,

in which the recoded E protein encoding sequence is about a continuous one-third to one-half of the length of an entire E protein encoding sequence,

15 in which the codon pair bias of the recoded E protein-encoding sequence is reduced by at least 0.05, and

substituting the recoded envelope (E) protein encoding sequence into the parent dengue virus genome to make a modified dengue virus genome; whereby expression of the recoded envelope (E) protein encoding sequence is reduced compared to the parent virus, in which the
20 parent E protein encoding sequence is from DENV-1/VN/BID-V1774/2007 (SEQ ID NO:1), DENV-2/NI/BID-V533/2005 (SEQ ID NO:3), DENV-3/VE/BID-V2268/2008 (SEQ ID NO:5), or DENV-4/US/BID-V2448/1999 (SEQ ID NO:7).

16. A modified dengue virus according to Claim 1, in which the modified dengue virus comprises a recoded sequence of SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID
25 NO:12.

17. A tetravalent dengue virus composition according to Claim 4, in which the parent E protein encoding sequence of serotype 1 is from DENV-1/VN/BID-V1774/2007 (SEQ ID NO:1), the parent E protein encoding sequence of serotype 2 is from DENV-2/NI/BID-V533/2005 (SEQ ID NO:3), the parent E protein encoding sequence of serotype 3 is from

DENV-3/VE/BID-V2268/2008 (SEQ ID NO:5), and the parent E protein encoding sequence of serotype 4 is from DENV-4/US/BID-V2448/1999 (SEQ ID NO:7).

18. A tetravalent dengue virus composition according to Claim 4, comprising modified dengue viruses having sequences of SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:11, and SEQ
5 ID NO:12.

19. A tetravalent dengue virus composition according to any of Claims 17 or 18 for use in eliciting an immune response in a human subject.

20. A modified dengue virus according to Claim 16 for use in eliciting an immune response in a human subject.