

CLAIMS

1. A nucleic acid construct encoding a modified genome or RNA replicon of an alphavirus species, wherein a first nucleic acid sequence encoding a portion of a nonstructural protein (nsP) of the modified alphavirus genome or RNA replicon is replaced with a second nucleic acid sequence encoding the corresponding nsP sequence derived from a different alphavirus strain, wherein said portion comprises at least 20% continuous residues of the amino acid sequence of said nsP.
2. The nucleic acid construct of claim 1, wherein the nsP is nsP1, nsP2, nsP3, or nsP4.
3. The nucleic acid construct of any one of claims 1-2, wherein the modified alphavirus genome or RNA replicon is devoid of at least a portion of the nucleic acid sequence encoding one or more viral structural proteins.
4. The nucleic acid construct of any one of claims 1-3, further comprising one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous nucleic acid sequence.
5. The nucleic acid construct of claim 4, wherein at least one of the expression cassettes comprises a subgenomic (*sg*) promoter operably linked to a heterologous nucleic acid sequence.
6. The nucleic acid construct of any one of claims 4-5, wherein at least one of expression cassettes comprises a coding sequence for a gene of interest (GOI).
7. The nucleic acid construct of any one of claims 1-6, wherein the nucleic acid construct is incorporated into a vector.
8. A recombinant cell comprising a nucleic acid construct according to any one of claims 1-7.
9. A cell culture comprising at least one recombinant cell according to claim 8, and a culture medium.
10. A method for functionalizing/engineering an alphavirus genome or RNA replicon, comprising:

- (a) providing a non-functional alphavirus genome or RNA replicon;
 - (b) replacing a first nucleic acid sequence encoding a portion of a nonstructural protein (nsP) of the non-functional alphavirus genome or RNA replicon with a second sequence nucleic acid sequence encoding the corresponding nsP sequence derived from a different alphavirus strain to generate a modified alphavirus genome or RNA replicon, wherein said portion comprises at least 20% continuous residues of the amino acid sequence of said nsP;
 - (c) assessing functionality of the modified alphavirus genome or RNA replicon;
 - (d) identifying the modified alphavirus genome or RNA replicon as being functional if the modified alphavirus genome or RNA replicon is capable of RNA replication and/or expression.
11. The method of claim 10, wherein the non-functionality of the alphavirus genome or RNA replicon is determined by a deficiency in self-replication within a host cell.
12. The method of any one of claims 10-11, wherein the assessing functionality of the modified alphavirus genome or RNA replicon comprises an assay selected from the group consisting of: detection of RNA replication, detection of viral protein expression, detection of cytopathic effect (CPE), and detection of heterologous transgene expression.
13. A transgenic non-human animal comprising a nucleic acid construct according to any one of claims 1-7.
14. A method for producing a polypeptide of interest, comprising (i) rearing a transgenic non-human animal comprising a nucleic acid construct according to any one of claims 6-7, or (ii) culturing a recombinant cell comprising a nucleic acid construct according to any one of claims 6-7 under conditions wherein the transgenic non-human animal or the recombinant cell produces the polypeptide encoded by the GOI, and wherein the nucleic acid construct comprises a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-4.
15. The method of claim 14, wherein the nucleic acid construct comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-4.

16. A non-therapeutic method for producing a polypeptide of interest in a non-human subject, comprising a nucleic acid construct according to any one of claims 6-7 to be introduced into the non-human subject, wherein the nucleic acid construct comprises a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-4.

17. The non-therapeutic method of claim 16, wherein the nucleic acid construct comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-4.

18. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and:
(a) a nucleic acid construct of any one of claims 6-7, wherein the nucleic acid construct comprises a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-4; and/or
(b) a recombinant cell of claim 8.

19. The pharmaceutical composition of claim 18, wherein the nucleic acid construct comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-4.

20. Use of a composition in the manufacture of a medicament for the induction of a pharmacodynamic effect, for preventing and/or treating a health condition in a subject in need thereof, wherein composition comprises:

- (a) a nucleic acid construct of any one of claims 6-7, wherein the nucleic acid construct comprises a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-4;
- (b) a recombinant cell of claim 8; and/or
- (c) a pharmaceutical composition of claim 18 or 19,

wherein the pharmacodynamic effect is an immune response and/or an induction of a cytokine pathway, and wherein:

- (i) the health condition is a rabies infection and the coding sequence for the GOI encodes an envelope glycoprotein of a rabies virus;
- (ii) the health condition is a proliferative disorder and the coding sequence for the GOI encodes ESR1, HER2, and HER3; or

(iii) the health condition is an inflammatory disorder and the coding sequence for the GOI encodes IL-RA1 and IL-12.

21. Use of a composition in the manufacture of a medicament for eliciting an immune response related to a health condition in a subject in need thereof, wherein the composition comprises:

- (a) a nucleic acid construct of any one of claims 6-7, wherein the nucleic acid construct comprises a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-4;
- (b) a recombinant cell of claim 8; and/or
- (c) a pharmaceutical composition of claim 18 or 19,

and wherein:

(i) the health condition is a rabies infection and the coding sequence for the GOI encodes an envelope glycoprotein of a rabies virus;

(ii) the health condition is a proliferative disorder and the coding sequence for the GOI encodes ESR1, HER2, and HER3; or

(iii) the health condition is an inflammatory disorder and the coding sequence for the GOI encodes IL-RA1 and IL-12.

22. The use of any one of claims 20-21, wherein the health condition is a rabies infection.

23. A kit for inducing a pharmacodynamic effect, for eliciting an immune response, for the prevention, and/or for the treatment of a health condition, the kit comprising:

- (a) a nucleic acid construct of any one of claims 6-7, wherein the nucleic acid construct comprises a nucleic acid sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-4;
- (b) a recombinant cell of claim 8; and/or
- (c) a pharmaceutical composition of claim 18 or 19,

wherein the pharmacodynamic effect is an immune response or an induction of a cytokine pathway;

and wherein:

- (i) the health condition is a rabies infection and the coding sequence for the GOI encodes an envelope glycoprotein of a rabies virus;
- (ii) the health condition is a proliferative disorder and the coding sequence for the GOI encodes ESR1, HER2, and HER3; or
- (iii) the health condition is an inflammatory disorder and the coding sequence for the GOI encodes IL-RA1 and IL-12.