Claims

1. A polynucleotide or a set of polynucleotides comprising a first nucleic acid molecule encoding an influenza non-structural (NS1) protein and a second nucleic acid molecule encoding a heterologous target mRNA, wherein the heterologous target mRNA encodes a biologically active polypeptide, which comprises a cytokine, a chemokine, a growth factor, a clotting factor, an enzyme, or any combination thereof.

2. The polynucleotide or set of polynucleotides of claim 1, wherein the first nucleic acid moleculeand the second nucleic acid molecule are present in a single vector or different vectors.

3. The polynucleotide or set of polynucleotides of claim 1 or 2, wherein the first nucleic acid moleculeis expressed under the control of a first promoter and the second nucleic acid molecule is expressed under the control of a second promoter, and wherein the first promoter and the second promoter are the same or different.

4. The polynucleotide or set of polynucleotides of any one of claims 1 to 3, wherein the expression of the target mRNA is increased relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein.

5. The polynucleotide or set of polynucleotides of any one of claims 1 to 4, wherein the cytokine comprises an IL-12 polypeptide or a fragment or variant thereof.

6. The polynucleotide or set of polynucleotides of any one of claims 3 to 5, wherein the first promoter, second promoter, or both comprise an inducible promoter, a tissue specific promoter, or a constitutively active promoter.

7. The polynucleotide or set of polynucleotides of any one of claims 1 to 6, wherein the influenza NS1 is a type A influenza virus NS1, a type B influenza virus NS1, a type C influenza virus NS1, or a variant thereof.

8. The polynucleotide or set of polynucleotides of any one of claims 1 to 7, wherein the influenza NS1 is an H1N1 NS1, H1N2 NS1, H2N2 NS1, H3N2 NS1, H5N1 NS1, H7N9 NS1, H7N7 NS1, H9N2 NS1, H7N2 NS1, H7N3 NS1, H5N2 NS1, H10N7 NS1, or any combination thereof.

9. The polynucleotide or set of polynucleotides of claim 8, wherein the H1N1 NS1 is the H1N1 TX91 variant NS1.

10. The polynucleotide or set of polynucleotides of any one of claims 1 to 9, wherein the first nucleic acid molecule comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1 or 2.

11. The polynucleotide or set of polynucleotides of any one of claims 1 to 10, comprising one or more modified nucleic acid molecule.

12. The polynucleotide or set of polynucleotides of any one of claims 1 to 11, wherein: (i) the first nucleic acid molecule, (ii) the second nucleic acid molecule, or (iii) both (i) and (ii) are circular RNA.

13. A polynucleotide or a set of polynucleotides comprising a self-replicating target mRNA, wherein the self-replicating target mRNA comprises one or more modified nucleic acid molecule.

14. The polynucleotide or set of polynucleotides of any one of claims 1 to 13, wherein about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules, and wherein the one or more modified nucleic acid molecule is a modified ribonucleotide triphosphate (rNTP).

15. The polynucleotide or the set of polynucleotides of claim 14, wherein the modified rNTP comprises N1-methylpsuedo uracil, 5-methyl cytosine, N6-methyladenosine, or combinations thereof.

16. A vector or a set of vectors comprising the polynucleotide or the set of polynucleotides of any one of claims 1 to 15.

17. The vector or the set of vectors of claim 16, which is a replicon, and wherein the replicon is a Venezuelan equine encephalitis (VEE) replicon comprising a nucleotide sequence encoding a lysine at residue 739, according to the wild-type amino acid sequence VEE.

18. The vector or the set of vectors of claim 16 or 17, wherein the vector comprises:

(a) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA; and (v) a VEE 3'UTR or a derivative thereof;

(b) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; and (vi) a VEE 3'UTR or a derivative thereof;

(c) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA; (v) an E1 sequence; and (vi) a VEE 3'UTR or a derivative thereof;

(d) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof;

(e) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof;

(f) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof;

(g) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a second nucleic acid molecule encoding the target mRNA; and (v) a VEE 3'UTR or a derivative thereof;

(h) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof;

(i) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof;

(j) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof;

(k) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof;

(l) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof;

(m) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof;

(n) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof;

(o) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 2; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof; or

(p) (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 2; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

19. A cell comprising the polynucleotide or set of polynucleotides of any one of claims 1 to 15 or the vector or the set of vectors of any one of claims 16 to 18.

20. A pharmaceutical composition comprising the polynucleotide or set of polynucleotides of any one of claims 1 to 15, the vector or the set of vectors of any one of claims 16 to 18, or the cell of claim 19and a pharmaceutically acceptable carrier.

21. A method of expressing a target mRNA in a cell, comprising transfecting the cell with the polynucleotide or set of polynucleotides of any one of claims 1 to 15 or the vector or the set of vectors of any one of claims 16 to 18.

22. The polynucleotide or set of polynucleotides of any one of claims 1 to 15, the vector or the set of vectors of any one of claims 16 to 18, the cell of claim 19, or the pharmaceutical composition of claim 20 for use in treating a subject in need thereof.

23. The polynucleotide or set of polynucleotides of any one of claims 1 to 15, the vector or the set of vectors of any one of claims 16 to 18, the cell of claim 19, or the pharmaceutical composition of claim 20 for use in expressing a target mRNA in a subject in need thereof.

24. The use of claim 22 or 23, wherein the subject is afflicted with a cancer.

25. A method of expressing a target mRNA in a cell, comprising co-expressing the target mRNA and an influenza NS1 protein in the cell, wherein the target mRNA is not an influenza mRNA.

26. A method of expressing a target mRNA in a cell, comprising transfecting the cell with a polynucleotide or a set of polynucleotides comprising a self-replicating target mRNA comprising one or more modified nucleic acid molecules.

27. The method of claim 26, wherein expression of the target mRNA is increased relative to the expression of the target mRNA from a self-replicating target mRNA not comprising one or more modified nucleic acid molecules.