CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid construct comprising a nucleic acid sequence encoding a modified Western equine encephalitis virus (WEEV) genome or self-replicating RNA (srRNA), wherein the modified WEEV genome or srRNA is devoid of at least a portion of the nucleic acid sequence encoding one or more viral structural proteins.

2. The nucleic acid construct of claim 1, wherein:

(a) the modified viral genome or srRNA is devoid of a substantial portion of the nucleic acid sequence encoding one or more viral structural proteins;

(b) the modified viral genome or srRNA comprises no nucleic acid sequence encoding viral structural proteins;

(c) the nucleic acid construct further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous nucleic acid sequence;

(d) the nucleic acid construct further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous nucleic acid sequence, and at least one of the expression cassettes comprises a subgenomic (sg) promoter operably linked to a heterologous nucleic acid sequence;

(e) the nucleic acid construct further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous nucleic acid sequence, at least one of the expression cassettes comprises a subgenomic (sg) promoter operably linked to a heterologous nucleic acid sequence, and the sg promoter is a 26S subgenomic promoter;

(f) at least one nonstructural protein (nsP), or a portion thereof, of the modified WEEV genome or srRNA is heterologous relative to the remainder of the modified WEEV genome or srRNA;

(g) the nucleic acid construct further comprises a nucleic acid sequence encoding a heterologous nsP or a portion thereof;

(h) the nucleic acid construct further comprises one or more untranslated regions (UTRs);

(i) the nucleic acid construct further comprises one or more untranslated regions (UTRs), and at least one of the UTRs is a heterologous UTR;

(j) the nucleic acid construct further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous

nucleic acid sequence, and at least one of the expression cassettes comprises a coding sequence for a gene of interest (GOI);

(k) the nucleic acid construct further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous nucleic acid sequence, and at least one of the expression cassettes comprises a coding sequence for a gene of interest (GOI), which encodes a polypeptide selected from the group consisting of a therapeutic polypeptide, a prophylactic polypeptide, a diagnostic polypeptide, a nutraceutical polypeptide, an industrial enzyme, and a reporter polypeptide;

(l) the nucleic acid construct further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous nucleic acid sequence, and at least one of the expression cassettes comprises a coding sequence for a gene of interest (GOI), which encodes a polypeptide selected from the group consisting of an antibody, an antigen, an immune modulator, an enzyme, a signaling protein, and a cytokine;

(m) the nucleic acid construct further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous nucleic acid sequence, at least one of the expression cassettes comprises a coding sequence for a gene of interest (GOI), and the coding sequence of the GOI is optimized for expression at a level higher than the expression level of a reference coding sequence;

(n) the nucleic acid construct further comprises one or more expression cassettes, wherein each of the expression cassettes comprises a promoter operably linked to a heterologous nucleic acid sequence, at least one of the expression cassettes comprises a coding sequence for a gene of interest (GOI), and the coding sequence of the GOI is optimized for enhanced RNA stability; and/or

(o) the nucleic acid sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

3. A recombinant cell comprising a nucleic acid construct according to claim 1 or claim 2, optionally wherein:

(a) the recombinant cell is a eukaryotic cell;

(b) the recombinant cell is an animal cell;

(c) the recombinant cell is a vertebrate animal cell or an invertebrate animal cell;

(d) the recombinant cell is an insect cell;

(e) the recombinant cell is a mosquito cell;

(f) the recombinant cell is a mammalian cell; or

(g) the recombinant cell is a vertebrate animal cell selected from the group consisting of a monkey kidney CV1 cell transformed by SV40 (COS-7), a human embryonic kidney cell (e.g., HEK 293 or HEK 293 cell), a baby hamster kidney cell (BHK), a mouse sertoli cell (e.g., TM4 cells), a monkey kidney cell (CV1), a human cervical carcinoma cell (HeLa), a canine kidney cell (MDCK), a buffalo rat liver cell (BRL 3A), a human lung cell (W138), a human liver cell (Hep G2), a mouse mammary tumor (MMT 060562), a TRI cell, a FS4 cell, a Chinese hamster ovary cell (CHO cell), an African green monkey kidney cell (Vero cell), a human A549 cell, a human cervix cell, a human CHME5 cell, a human PER.C6 cell, a NS0 murine myeloma cell, a human epidermoid larynx cell, a human fibroblast cell, a human HUH-7 cell, a human MRC-5 cell, a human muscle cell, a human endothelial cell, a human astrocyte cell, a human macrophage cell, a human RAW 264.7 cell, a mouse 3T3 cell, a mouse L929 cell, a mouse connective tissue cell, a mouse muscle cell, and a rabbit kidney cell.

4. A cell culture comprising at least one recombinant cell according to claim 3, and a culture medium.

5. A transgenic animal comprising a nucleic acid construct according to claim 1 or claim 2.

6. The transgenic animal of claim 5, wherein:

(a) the animal is a vertebrate animal or an invertebrate animal;

(b) the animal is an insect;

(c) the animal is a mammalian; or

(d) the animal is a non-human mammalian.

7. A method for producing a polypeptide of interest, comprising:

(a) (i) rearing a transgenic animal according to claim 5 or claim 6, or (ii) culturing a recombinant cell comprising a nucleic acid construct according to any one of claims 2(k) to 2(o) under conditions wherein the transgenic animal or the recombinant cell produces the polypeptide encoded by the GOI; or

(b) administering to the subject a nucleic acid construct according to any one of claims 2(k) to 2(o).

8. The method of claim 7, wherein:

(a) the subject is vertebrate animal or an invertebrate animal;
(b) the subject is an insect;
(c) the subject is a mammalian subject; or
(d) the subject is a human subject.

9. A recombinant polypeptide produced by the method of claim 7 or claim 8.

10. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and:
a) a nucleic acid construct of claim 1 or claim 2;
b) a recombinant cell of claim 3; and/or
c) a recombinant polypeptide of claim 9.

11. The pharmaceutical composition of claim 10, wherein:
(a) the pharmaceutical composition comprises:
(i) a nucleic acid construct of claim 1 or claim 2, and a pharmaceutically acceptable excipient;
(ii) a recombinant cell of claim 3, and a pharmaceutically acceptable excipient;
(iii) a recombinant polypeptide of claim 9, and a pharmaceutically acceptable excipient;
(b) the composition is formulated in a liposome, a lipid-based nanoparticle (LNP), a polymer nanoparticle, a polyplex, a viral replicon particle (VRP), a microsphere, an immune stimulating complex (ISCOM), a conjugate of bioactive ligand, or a combination of any thereof;
(c)
(i) the composition is an immunogenic composition;
(ii) the composition is an immunogenic composition which is formulated as a vaccine; or
(iii) the composition is substantially non-immunogenic to a subject;
(d) the pharmaceutical composition is formulated as an adjuvant; and/or
(e) the pharmaceutical composition is formulated for one or more of intranasal administration, intrathecal administration, transdermal administration, intraperitoneal administration, intramuscular administration, intratracheal administration, intranodal administration, intratumoral administration, intraarticular administration, intravenous administration, subcutaneous administration, intravaginal administration, intraocular administration, rectal administration, and oral administration.

12. The nucleic acid construct of claim 1 or claim 2, the recombinant cell of claim 3, the recombinant polypeptide of claim 9 or the pharmaceutical composition of claim 10 or claim 11 for use in :

(i) eliciting a pharmacodynamic effect in a subject in need thereof, wherein the subject is to be administered a composition comprising:

a) the nucleic acid construct;

b) the recombinant cell;

c) the recombinant polypeptide; and/or

d) the pharmaceutical composition; or

(ii) preventing and/or treating a health condition in a subject in need thereof, wherein the subject is to be administered, prophylactically or therapeutically, a composition comprising:

a) the nucleic acid construct;

b) the recombinant cell;

c) the recombinant polypeptide; and/or

d) the pharmaceutical composition.

13. The nucleic acid construct, recombinant cell, recombinant polypeptide or pharmaceutical composition for use according to claim 12(i), wherein the pharmacodynamic effect comprises one or more of the following: immunogenicity effect, a biomarker response, a therapeutic effect, a prophylactic effect, a desired effect, an undesired effect, an adverse effect, and effect in a disease model, optionally wherein the pharmacodynamic effect comprises eliciting an immune response in the subject.

14. The nucleic acid construct, recombinant cell, recombinant polypeptide or pharmaceutical composition for use according to claim 12(ii), wherein:

(a) the administered composition elicits an immune response in the subject;

(b) the condition is a proliferative disorder or a microbial infection;

(c) the subject has or is suspected of having a condition associated with proliferative disorder or a microbial infection;

(d) the administered composition results in an increased production of interferon in the subject;

(e) the composition is to be administered to the subject individually as a single therapy (monotherapy) or as a first therapy in combination with at least one additional therapies;

and/or

(f) the composition is to be administered to the subject as a first therapy in combination with at least one additional therapies, and the at least one additional therapies is selected from the group consisting of chemotherapy, radiotherapy, immunotherapy, hormonal therapy, toxin therapy, targeted therapy, and surgery.

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

a) a nucleic acid construct of claim 1 or claim 2;

b) a recombinant cell of claim 3;

c) a recombinant polypeptide of claim 9; and/or

d) a pharmaceutical composition of claim 10 or claim 11,

and instructions for use according to one of claims 12 to 14.