CLAIMS

1. A method for identifying and/or characterizing a self-replicating RNA (srRNA) construct, the method comprising:

(a) providing a plurality of srRNA expression constructs each comprising a coding sequence for a polypeptide construct of interest (PCI) operably inserted into an alphavirus srRNA vector, wherein at least a portion of the coding sequence for the alphavirus structural proteins has been replaced with the coding sequence of the PCI;

(b) analyzing level and/or functionality of the PCIs that are expressed from the plurality of srRNA expression constructs to identify one or more candidate PCIs having a defined property;

(c) incorporating the srRNA expression constructs capable of expressing the candidate PCIs identified in (b) with at least one delivery vehicle to create a combinatorial collection of delivery systems; and

(d) analyzing the delivery systems for their capability to confer at least one pharmacodynamic effect in a subject to identify a srRNA expression construct capable of conferring a desired pharmacodynamic effect.

2. The method of claim 1, wherein:

(i) the coding sequence for the PCI comprises a coding sequence for a single polypeptide or coding sequences for a plurality of polypeptides;

(ii) the coding sequence for the PCI comprises coding sequences for a plurality of polypeptides wherein the coding sequences of the plurality of polypeptides are operably linked to one another within a single open reading frame (i.e., in a polycistronic ORF);

(iii) the coding sequence for the PCI comprises coding sequences for a plurality of polypeptides wherein the plurality of polypeptides are operably linked to one another by one or more connector sequences;

(iv) the coding sequence for the PCI comprises coding sequences for a plurality of polypeptides wherein the plurality of polypeptides are operably linked to one another by one or more connector sequences, and a connector sequence of the plurality of connector sequences comprises an autoproteolytic peptide sequence;

(v) the coding sequence for the PCI comprises coding sequences for a plurality of polypeptides, wherein the plurality of polypeptides are operably linked to one another by one or more connector sequences, a connector sequence of the plurality of connector sequences comprises an autoproteolytic peptide sequence, and the

autoproteolytic peptide sequence comprises one or more autoproteolytic cleavage sequences derived from a calcium-dependent serine endoprotease (furin), a porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof;

(vi) the coding sequence for the PCI comprises coding sequences for a plurality of polypeptides, wherein the coding sequences of the plurality of polypeptides are operably linked to one another by one or more internal ribosomal entry sites (IRES);

(vii) the coding sequence for the PCI comprises coding sequences for a plurality of polypeptides, wherein the coding sequences of the plurality of polypeptides are operably linked to one another by one or more internal ribosomal entry sites (IRES), and the one or more IRES is selected from a viral IRES, a cellular IRES, and an artificial IRES; and/or

(viii) the coding sequence for the PCI comprises coding sequences for a plurality of polypeptides, wherein the coding sequences of the plurality of polypeptides are operably linked to one another by one or more internal ribosomal entry sites (IRES), and the one or more IRES is selected from a Kaposi's sarcoma-associated herpesvirus (KSHV) IRES, a hepatitis virus IRES, a Pestivirus IRES, a Cripavirus IRES, a Rhopalosiphum padi virus IRES, a fibroblast growth factor IRES, a platelet-derived growth factor IRES, a vascular endothelial growth factor IRES, an insulin-like growth factor IRES, a picornavirus IRES, an encephalomyocarditis virus (EMCV) IRES, a Pim-1 IRES, a p53 IRES, an Apaf-1 IRES, a TDP2 IRES, an L-myc IRES, and a c-myc IRES.

3. The method of claim 1 or claim 2, wherein:

(i) the PCI comprises one or more polypeptides selected from microbial proteins, viral proteins, bacterial proteins, fungal proteins, mammalian proteins, and combinations of any thereof;

(ii) the PCI comprises one or more polypeptides selected from antigen molecules, biotherapeutic molecules, or combinations of any thereof;

(iii) the PCI comprises one or more antigen polypeptides selected from tumor-associated antigens, tumor-specific antigens, neoantigens, and combinations of any thereof;

(iv) the PCI comprises one or more antigen polypeptides, wherein the one or more antigen polypeptides comprises estrogen receptors, intracellular signal transducer enzymes, and human epidermal growth receptors;

(v) the PCI comprises one or more antigen polypeptides, wherein the one or more antigen polypeptides is selected from ESR1, PI3K, HER2, HER3, variants of any thereof, and combinations of any thereof;

(vi) the PCI comprises one or more biotherapeutic polypeptides selected from immunomodulators, modulators of angiogenesis, modulators of extracellular matrix, modulators of metabolism, neurological modulators, and combinations of any thereof;

(vii) the PCI comprises one or more cytokines selected from chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors;

(viii) the PCI comprises one or more interleukins selected from IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, IL-15, IL-17, IL-23, IL-27, IL-35, IFNγ and subunits of any thereof; and/or

(ix) the PCI comprises one or more biotherapeutic polypeptides, wherein the one or more biotherapeutic polypeptides is selected from IL-12A, IL-12B, IL-1RA, and combinations of any thereof.

4. The method of any one of claims 1 to 3, wherein step (a) comprises providing a plurality of srRNA expression constructs each comprising a coding sequence for a variant of the PCI.

5. The method of claim 4, wherein:

(I) the providing in (a) comprises:

i) obtaining an alphavirus srRNA expression vector, wherein at least a portion of encoding sequence for the alphavirus structural proteins has been replaced with a coding sequence for a polypeptide construct of interest (PCI); and

ii) generating a plurality of srRNA expression constructs each comprising a coding sequence for a variant of the PCI; or

(II) the providing in (a) comprises:

i) obtaining coding sequences for a plurality of variants of a polypeptide construct of interest (PCI); and

ii) generating a plurality of srRNA expression constructs each comprising a coding sequence for a PCI variant of the plurality of PCI variants from (a) operably inserted into an alphavirus srRNA vector, wherein at least a portion of the encoding sequence for the alphavirus structural proteins has been replaced with the coding sequence of the PCI variant.

6. The method of claim 5, wherein:

(i) a PCI variant of the plurality of PCI variants comprises one or more molecular alterations;

(ii) a PCI variant of the plurality of PCI variants comprises one or more molecular alterations, wherein the one or more molecular alterations in the PCI variant is selected from the group consisting of deletions, substitutions, insertion, duplications, mutations, frameshift variants, splice variants, and combinations of any thereof;

(iii) a PCI variant of the plurality of PCI variants comprises one or more molecular alterations, wherein the one or more molecular alterations are configured into a plurality of alteration cassettes arranged in tandem along the length of the antigen sequence;

(iv) a PCI variant of the plurality of PCI variants comprises one or more molecular alterations, configured into a plurality of alteration cassettes arranged in tandem along the length of the antigen sequence, wherein an alteration cassette of the plurality of alteration cassettes comprises one, two, three, four, five, or more molecular alterations;

(v) a PCI variant of the plurality of PCI variants comprises one or more molecular alterations, wherein the one or more molecular alterations are configured into a plurality of alteration cassettes arranged in tandem along the length of the antigen sequence, wherein the plurality of alteration cassettes are operably linked to one another by one or more linkers;

(vi) a PCI variant of the plurality of PCI variants comprises one or more molecular alterations, wherein the one or more molecular alterations are configured into a plurality of alteration cassettes arranged in tandem along the length of the antigen sequence, wherein the plurality of alteration cassettes are operably linked to one another by one or more linkers, wherein a linker of the one or more linkers comprises a synthetic compound linker or a peptide linker; and/or

(vii) a PCI variant of the plurality of PCI variants comprises one or more molecular alterations, wherein the one or more molecular alterations are configured into a plurality of alteration cassettes arranged in tandem along the length of the antigen sequence, wherein the plurality of alteration cassettes are operably linked to one another by one or more linkers, wherein a linker of the one or more linkers comprises a synthetic compound linker or a peptide linker, wherein the peptide linker comprises an amino acid sequence selected from the group consisting of AAY, EAAAK (SEQ ID NO: 1), RVRR (SEQ ID NO: 2), GGGGS (SEQ ID NO: 3), and GPGPG (SEQ ID NO: 4).

7. The method of any one of claims 1 to 6, wherein:
(i) the analyzing level and/or functionality of the PCIs in step (b) is carried out in vitro, in vivo, or ex vivo;
(ii) the analyzing level and/or functionality of the PCIs in step (b) comprises immunoblotting analysis, fluorescence flow cytometry analysis, enzyme-linked immunoassay analysis, immunogenicity analysis, bioactivity analysis, and/or efficacy in a disease model;
(iii) the analysis of the delivery systems for theirs capacity to confer at least one pharmacodynamic effects in step (d) is carried out in vivo or ex vivo;
(iv) the at least one pharmacodynamic effects 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; and/or
(v) the at least one pharmacodynamic effects comprises induction of an immune response.

8. The method of any one of claims 1 to 7, wherein:
(i) the delivery systems comprise a physiologic buffer, a liposome, a lipid-based nanoparticle (LNP), a polymer nanoparticle, a viral replicon particle (VRP), a microsphere, an immune stimulating complex (ISCOM), a conjugate of bioactive ligand, or a combination of any thereof;
(ii) the delivery systems comprise a lipid-based nanoparticle (LNP), wherein the LNP delivery system comprises:
(I) a cationic lipid, an ionizable cationic lipid, an anionic lipid, or a neutral lipid;
(II) an ionizable cationic lipid selected from the group consisting of ALC-0315, C12-200, LN16, MC3, MD1, SM-102, and a combination of any thereof;
(III) a cationic lipid selected from the group consisting of 98N12-5, C12-200, C14-PEG2000, DLin-KC2-DMA (KC2), DLin-MC3-DMA (MC3), XTC, MD1, 7C1, and a combination of any thereof;
(IV) a neutral lipid selected from the group consisting of DPSC, DPPC, POPC, DOPE, SM, and a combination of any thereof; or
(V) lipid selected from the group consisting of C12-200, C14-PEG2000, DOPE, DMG-PEG2000, DSPC, DOTMA, DOSPA, DOTAP, DMRIE, DC-cholesterol, DOTAP–cholesterol, GAP-DMORIE–DPyPE, and GL67A–DOPE–DMPE–polyethylene glycol (PEG); and/or

(iii) the delivery systems comprise a lipid-based nanoparticle (LNP), wherein:

(I) the mass ratio of lipid to nucleic acid in the LNP delivery system is:

(A) about 100:1 to about 3:1, about 70:1 to 10:1, or 16:1 to 4:1;

(B) about 16:1 to 4:1;

(C) about 20:1; or

(D) about 8:1; and/or

(II) the lipid-based nanoparticles (LNPs) have an average diameter:

(A) of less than about 1000 nm, about 500 nm, about 250 nm, about 200 nm, about 150 nm, about 100 nm, about 75 nm, about 50 nm, or about 25 nm;

(B) ranging from about 70 nm to 100 nm; or

(C) ranging from about 88 nm to about 92 nm, from 82 nm to about 86 nm, or from about 80 nm to about 95 nm.

9. The method of any one of claims 1 to 8, wherein:

(i) the recombinant alphavirus srRNA is of a virus belonging to the Alphavirus genus of the Togaviridae family;

(ii) the recombinant alphavirus srRNA is of an alphavirus belonging to the VEEV/EEEV group, or the SFV group, or the SINV group;

(iii) the recombinant alphavirus srRNA is of an alphavirus belonging to the VEEV/EEEV group, or the SFV group, or the SINV group, wherein the alphavirus is Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus (AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu virus (NDUV), Madariaga virus (MADV), or Buggy Creek virus;

(iv) the recombinant alphavirus srRNA is of an alphavirus belonging to the VEEV/EEEV group, or the SFV group, or the SINV group, wherein the alphavirus is Venezuelan equine encephalitis virus (VEEV), Eastern Equine Encephalitis virus (EEEV), Chikungunya virus (CHIKV), or Sindbis virus (SINV); and/or

(v) the srRNA expression vector is identified as having an immune-inducing activity suitable for a prophylactic use and/or a therapeutic use.

10. A self-replicating RNA (srRNA) construct identified according to a method of claims 1 to 9, a nucleic acid encoding the srRNA construct, a recombinant cell comprising the self-replicating RNA construct and/or the nucleic acid encoding the srRNA construct, a cell culture comprising the recombinant cell and a cell culture medium, or a composition comprising a therapeutically acceptable excipient and the srRNA construct, the nucleic acid, or the recombinant cell.

11. The recombinant cell of claim 10, wherein the recombinant cell is:
(i) a eukaryotic cell;
(ii) an animal cell;
(iii) a vertebrate animal cell or an invertebrate animal cell;
(iv) a mammalian cell;
(v) an insect cell;
(vi) a mosquito cell;
(vii) an immune cell;
(viii) an immune cell, wherein the immune cell is a B cell, a monocyte, a natural killer (NK) cell, a natural killer T (NKT) cell, a basophil, an eosinophil, a neutrophil, a dendritic cell (DC), a macrophage, a regulatory T cell, a helper T cell (TH), a cytotoxic T cell (TCTL), a memory T cell, a gamma delta (γδ) T cell, a hematopoietic stem cell, or a hematopoietic stem cell progenitor; or
(ix) an immune cell, wherein the immune cell is a B cell, a T cell, or a dendritic cell (DC).

12. The composition of claim 10, wherein the composition is:
(i) formulated as a vaccine;
(ii) formulated as a vaccine, wherein the vaccine is a therapeutic vaccine;
(iii) formulated as an immunogenic composition;
(iv) formulated as a biotherapeutic;
(v) formulated with a delivery vehicle into a delivery system;
(vi) formulated with a delivery vehicle into a delivery system, wherein the delivery system comprises a physiologic buffer, a liposome, a lipid-based nanoparticle (LNP), a polymer nanoparticle, a viral replicon particle (VRP), a microsphere, an immune stimulating complex (ISCOM), a conjugate of bioactive ligand, or a combination of any thereof;
(vii) formulated with a delivery vehicle into an LNP delivery system, wherein the LNP delivery system comprises a cationic lipid, an ionizable cationic lipid, an anionic lipid, or a neutral lipid;

(viii) formulated with a delivery vehicle into an LNP delivery system, wherein the LNP comprises an ionizable cationic lipid selected from the group consisting of ALC-0315, C12-200, LN16, MC3, MD1, SM-102, and a combination of any thereof;

(ix) formulated with a delivery vehicle into an LNP delivery system, wherein the LNP comprises a cationic lipid selected from the group consisting of 98N12-5, C12-200, C14-PEG2000, DLin-KC2-DMA (KC2), DLin-MC3-DMA (MC3), XTC, MD1, 7C1, and a combination of any thereof;

(x) formulated with a delivery vehicle into an LNP delivery system, wherein the LNP comprises a neutral lipid selected from the group consisting of DPSC, DPPC, POPC, DOPE, SM, and a combination of any thereof;

(xi) formulated with a delivery vehicle into an LNP delivery system, wherein the LNP comprises a lipid selected from the group consisting of C12-200, C14-PEG2000, DOPE, DMG-PEG2000, DSPC, DOTMA, DOSPA, DOTAP, DMRIE, DC-cholesterol, DOTAP–cholesterol, GAP-DMORIE–DPyPE, GL67A–DOPE–DMPE–polyethylene glycol (PEG), and a combination of any thereof;

(xii) formulated with a delivery vehicle into an LNP delivery system, wherein the mass ratio of lipid to nucleic acid in the LNP delivery system is:

(I) about 100:1 to about 3:1, about 70:1 to 10:1, or 16:1 to 4:1;

(II) about 16:1 to 4:1;

(III) about 20:1; or

(IV) about 8:1; and/or

(xiii) formulated with a delivery vehicle into an LNP delivery system, wherein the lipid-based nanoparticles (LNPs) have an average diameter:

(I) of less than about 1000 nm, about 500 nm, about 250 nm, about 200 nm, about 150 nm, about 100 nm, about 75 nm, about 50 nm, or about 25 nm;

(II) ranging from about 70 nm to 100 nm; or

(III) ranging from about 88 nm to about 92 nm, from 82 nm to about 86 nm, or from about 80 nm to about 95 nm.

13. Use of the self-replicating RNA construct, the nucleic acid, the recombinant cell or the composition of any one of claims 10-12 in the manufacture of a medicament for:

(i) inducing a pharmacodynamic effect in a subject, wherein a composition comprising the self-replicating RNA construct, the nucleic acid, the recombinant cell or the composition is to be administered to the subject; or

(ii) for preventing or treating a health condition in a subject, wherein a composition comprising the self-replicating RNA construct, the nucleic acid, the recombinant cell or the composition is to be prophylactically or therapeutically administered to the

subject.

14. The use according to claim 13, wherein:
(i) the administered composition induces production of one or more pro-inflammatory molecules in the subject;
(ii) the administered composition induces production of one or more pro-inflammatory molecules in the subject, wherein the one or more pro-inflammatory molecules comprises interferon gamma (IFNγ), cytokines, TNF-α, GM-CSF, and MIP1α, granzyme B, granzyme A, perforin, or a combination of any thereof;
(iii) the subject has been previously treated with one or more therapies and has developed at least a partial resistance to said one or more therapies;
(iv) the subject has been previously treated with one or more therapies and has developed at least a partial resistance to said one or more therapies, wherein at least one of the one or more therapies comprises a small molecule;
(v) the health condition is a proliferative disorder, inflammatory disorder, autoimmune disorder, or a microbial infection;
(vi) the subject has or is suspected of having a health condition associated with a proliferative disorder, an inflammatory disorder, an autoimmune disorder, or a microbial infection;
(vii) 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
(viii) 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, wherein 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 use in the prevention and/or treatment of a health condition, the kit comprising one or more of the following:

(a) a self-amplifying RNA construct according to claim 10;
(b) a nucleic acid according to claim 10;
(c) a recombinant cell according to any one of claims 10 or 11; and
(d) a pharmaceutical composition according to any one of claims 10 or 12.

16. The self-replicating RNA construct, the nucleic acid, the recombinant cell or the composition of any one of claims 10-12 for use as a medicament.