

COMPOSITIONS AND METHODS FOR INDUCING IMMUNE RESPONSE AGAINST EPSTEIN-BARR VIRUSES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Serial No. 63/615,206, filed on December 27, 2023. The disclosure of the above-referenced application is herein expressly incorporated by reference in its entirety, including any drawings.

INCORPORATION OF THE SEQUENCE LISTING

[0002] The material in the accompanying Sequence Listing is hereby incorporated by reference into this application. The accompanying Sequence Listing XML file, named 058462_519001WO_SequenceListing.XML, was created on December 19, 2024, and is approximately 266,240 bytes in size.

FIELD

[0003] The present disclosure relates to the field of molecular virology and immunology, and particularly relates to nucleic acid molecules, *e.g.*, self-replicating RNA (srRNA) expressing antigenic determinant(s) derived from Epstein-Barr virus (EBV), pharmaceutical compositions and recombinant cells containing the same, as well as the use of such srRNA molecules, recombinant cells, and compositions for eliciting a pharmacodynamic effect in a subject. Also provided are methods for preventing and/or treating various health conditions associated with EBV infection.

BACKGROUND

[0004] Epstein-Barr virus (EBV) infects most people worldwide and persists for life due to complicated interplay between lytic infection and multiple types of latent infections. While usually asymptomatic, EBV is a causative agent in several types of cancer and has a strong association with autoimmune and inflammatory disease, such as multiple sclerosis. In particular, it is one of the most common human viruses that infects about 95% of the adult population worldwide and has been known to be associated with two B-cell lymphomas, Burkitt's and Hodgkin's lymphomas. EBV can also infect epithelial cells and is associated with nasopharyngeal cancer. EBV is spread through bodily fluids, most commonly saliva, and contracted primarily by young children and adolescents (approximately 50% and approximately 89% seropositivity, respectively). It is a major

cause of infectious mononucleosis (IM) in the U.S., accounting for over 90% of the approximately 1-2 million cases annually. Infectious mononucleosis can debilitate patients for weeks to months and, in some cases, can lead to hospitalization and splenic rupture. EBV infection is associated with the development and progression of certain lymphoproliferative disorders, cancers, and an increased risk of autoimmune diseases including multiple sclerosis, an autoimmune disease of the central nervous system.

[0005] Currently there are no FDA approved vaccines against EBV on the market. Therefore, there is a pressing need in the art for developing methods and agents, *e.g.*, prophylactic vaccines and therapeutic vaccines against EBV and EBV-associated diseases. The technical problem underlying the present disclosure is to identify alternative and/or improved compositions, means, and methods that allow therapeutic and preventive vaccination against EBV infection and EBV-associated diseases, such as infectious mononucleosis, multiple sclerosis, and EBV-positive cancers.

SUMMARY

[0006] The present disclosure relates generally to the development of immuno-therapeutics, such as recombinant nucleic acids constructs and pharmaceutical compositions including the same for use in the prevention and management of various health conditions such as proliferative disorders, inflammatory diseases, autoimmune diseases, and cancers. In particular, as described in greater detail below, some embodiments of the disclosure provide nucleic acid constructs containing sequences that encode a modified genome or self-replicating RNA (srRNA), *e.g.*, replicons, of an alphavirus in which at least a portion of the nucleic acid sequence encoding the viral structural proteins of the modified alphavirus genome or srRNA has been replaced by coding sequences for a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV) proteins. Also disclosed are recombinant cells and transgenic animals that have been engineered to include one or more of the nucleic acid constructs disclosed herein, pharmaceutical compositions including one or more of the following: (a) a nucleic acid construct of the disclosure, and (b) a recombinant cell of the disclosure. Also provided herein, in some embodiments, are EBV vaccines that elicit anti-EBV antibody responses, and/or potent neutralizing antibody responses, and/or robust anti-EBV T cell responses. Further provided in particular aspects of the disclosure are compositions and methods for eliciting a pharmacodynamic

effect in a subject in need thereof, and/or for the prevention and/or treatment of various health conditions, including inflammatory diseases, autoimmune diseases, proliferative disorders (*e.g.*, cancers) and viral infections.

[0007] In one aspect of the disclosure, provided herein are nucleic acid constructs including a nucleic acid sequence encoding a modified alphavirus genome or self-replicating RNA (srRNA), wherein at least a portion of the nucleic acid sequence encoding the viral structural proteins of the modified alphavirus genome or srRNA has been replaced by coding sequences for a polypeptide construct including one or more of antigenic determinants derived from Epstein-Barr virus (EBV).

[0008] Non-limiting exemplary embodiments of the nucleic acid constructs of the disclosure can include one or more of the following features. In some embodiments, one or more of antigenic determinants is derived from one or more EBV polypeptides/antigens encoded by a lytic gene, a pre-latent gene, a latent gene, an early gene, an immediate-early gene, a late gene, a reactivation from latency gene, a variant of any thereof, or a combination of any thereof. In some embodiments, the EBV latent gene is LMP2, BKRF1, BYRF1, BLRF3/BERF1, BERF2a/b, BERF3/4, BamHI-W, BNLF1 (LMP1), BARTs, or EBER1/2. In some embodiments, the EBV latent gene is LMP2, BNLF1 (LMP1), BKRF1 (EBNA1), BLRF3/BERF1 (EBNA3A-C, -LP), or BYRF1 (EBNA2). In some embodiments, the EBV immediate-early gene is BZLF1, BRLF1, or BMLF1. In some embodiments the early gene is BRRF1, BORF2, BaRF1, BXLf1, BGLF5, BLLF3, BKRF3, BALF5, BMRF1, BALF2, BSLF1, BBLF2/3, BBLF4, BSLF2, BHRF1, BALF1, BARF1, BGLF4, BFRF1, BHLF1, BHLF2, or BNLF2a. In some embodiments, the EBV late gene is BLLF1 (gp350/220), BNRF1, BPLF1, BOLF1, BVRF1, BBLF1, BGLF1, BSRF1, BRRF2, BDLF2, BKRF4, BcLF1, BDLF1, BFRF3, BLRF2, BdRF1, BBRF1, BVRF2, BGLF2, BORF1, BLRF1 (gN), BLLF1 (gp350/220), BZLF2, BKRF2 (gp25, gL), BBRF3 (gM), BXLf2 (gp85, gH), BILF1, BILF2, BALF4 (gp100, gB), BDLF3, BMRF2, BALF3, or BCRF1. In some embodiments, the EBV lytic gene is BLLF1 (gp350/220), BXLf2 (gp85, gH), BALF4 (gp100, gB), BSLF1 (gp42), or BKRF2 (gp25, gL). In some embodiments, the EBV pre-latent gene is BYRF1 (EBNA2). In some embodiments, one or more EBV genes is selected from the group consisting of BLLF1 (gp350/220), BZLF1, BXLf2 (gp85, gH), BKRF2 (gp25, gL), LMP2, and a combination of any thereof. In some embodiments, the LMP2 gene encodes for the isoform LMP2A. In some embodiments, the LMP2 gene encodes for the isoform LMP2B. The amino acid sequence of

LMP2B lacks exon 1 but otherwise overlaps with LMP2A.

[0009] In some embodiments of the disclosure, the coding sequences for the polypeptide construct includes coding sequences for the following genes: (a) BZLF1 or a variant thereof; (b) BLLF1 (gp350/220) or a variant thereof; (c) LMP2B or a variant thereof; (d) gH and gL, or a variant of any thereof; (e) BZLF1, LMP2B, gH, gL, and BLLF1 (gp350/220), or a variant of any thereof; (f) gH, gL, and BLLF1 (gp350/220), or a variant thereof; or (g) BZLF1 and LMP2B, or a variant of any thereof. In some embodiments, the BLLF1 (gp350/220) or variant thereof is devoid of a functional transmembrane (TM) domain. In some embodiments, the gH or variant thereof is devoid of a functional transmembrane (TM) domain. In some embodiments, the BZLF1 or variant thereof is devoid of (i) a functional nuclear localization sequence (NLS), and/or (ii) a functional transactivation (TA) domain.

[0010] In some embodiments, the polypeptide construct includes an amino acid sequence having 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 an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-34.

[0011] In some embodiments of the disclosure, the coding sequences for the antigenic determinants are operably linked to one another within a single open reading frame (*i.e.*, in a polycistronic ORF). In some embodiments of the disclosure, the coding sequences for the antigenic determinants are operably linked to one another within two or more ORFs. In some embodiments, the coding sequences for the antigenic determinants are operably linked to one another by one or more linkers. In some embodiments, the one or more linkers includes coding sequences for an autoproteolytic peptide or an internal ribosomal entry site (IRES). In some embodiments, the autoproteolytic peptide includes one or more autoproteolytic cleavage sequences 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 Thossea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof). In some embodiments, the polypeptide constructs of the disclosure include one or more P2A autoproteolytic cleavage sequences. In some embodiments, the internal ribosomal entry site (IRES) is 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. In some embodiments, the internal ribosomal entry site (IRES) is from encephalomyocarditis virus (EMCV).

[0012] In some embodiments, the one or more of EBV antigenic determinants is of an EBV type 1 (EBV-1), or an EBV type 2 (EBV-2), or a combination thereof. In some embodiments, the one or more of EBV antigenic determinants is of a virulent EBV strain or an avirulent EBV strain. In some embodiments, the one or more of EBV antigenic determinants is derived from an AG876 strain, an Akata strain, an Alaska strain, a B95-8 strain, a China 2 strain, a CVI 988 strain, a GD1 strain, a GD2 strain, a GP202 strain, a Hina 1 strain, a HKNPC strain, a M81 strain, a Mediterranean strain, a Mutu strain, a P3HR1 strain, a Raji strain, a SNU-719 strain, a YCCEL1 strain, or a combination thereof.

[0013] In some embodiments, the modified alphavirus genome or srRNA includes no nucleic acid sequence encoding viral structural proteins. In some embodiments, the nucleic acid sequence encoding the polypeptide construct is operably linked to a promoter sequence. In some embodiments, the promoter sequence is a 26S subgenomic (*sg*) promoter. In some embodiments, the srRNA is a capped srRNA comprising a 5'-cap. In some embodiments, the capped srRNA is a co-transcriptionally capped srRNA. In some embodiments, the capped srRNA is an enzymatically capped srRNA.

[0014] In some embodiments, the modified alphavirus genome or srRNA is of an alphavirus belonging to the VEEV/EEEV group, or the SFV group, or the SINV group. In some embodiments, the alphavirus is Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV), Chikungunya virus (CHIKV), Madariaga virus (MADV), Western equine encephalitis virus (WEEV), or Sindbis virus (SINV).

[0015] In some embodiments, 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 a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 35-56.

[0016] In one aspect, provided herein are recombinant cells including a nucleic acid construct as described herein. In a related aspect, provided herein are cell cultures including at

least one recombinant cell as described herein and a culture medium. Non-limiting exemplary embodiments of the recombinant cells of the disclosure can include one or more of the following features. In some embodiments, the recombinant cell is a eukaryotic cell. In some embodiments, the recombinant cell is an animal cell. In some embodiments, the animal cell is a vertebrate animal cell or an invertebrate animal cell. In some embodiments, the recombinant cell is an insect cell. In some embodiments, the recombinant insect cell is a mosquito cell. In some embodiments, the recombinant cell is a mammalian cell. In some embodiments, the recombinant cell is selected from the group consisting of a monkey kidney CV1 cell transformed by SV40, a human embryonic kidney cell (HEK), a baby hamster kidney cell (BHK) or a derivative cell thereof, a mouse sertoli cell, a monkey kidney cell, a human cervical carcinoma cell, a canine kidney cell, a buffalo rat liver cell, a human lung cell, a human liver cell, a mouse mammary tumor, a TRI cell, a FS4 cell, a Chinese hamster ovary cell (CHO), an African green monkey kidney 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 B cell, a human epithelial cell, a human T cell, a human dendritic 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.

[0017] In another aspect, provided herein are transgenic animals including a nucleic acid construct and/or a recombinant cell as described herein. In some embodiments, the transgenic animal is a vertebrate animal or an invertebrate animal. In some embodiments, the animal is an insect. In some embodiments, the animal is a mammalian. In some embodiments, the mammalian is a non-human mammalian.

[0018] In one aspect, provided herein are compositions, *e.g.*, pharmaceutical compositions including a pharmaceutically acceptable excipient and one or more of the following: (a) a nucleic acid construct as described herein; and (b) a recombinant cell as described herein.

[0019] Non-limiting exemplary embodiments of the pharmaceutical compositions of the disclosure can include one or more of the following features. In some embodiments, the composition includes a nucleic acid construct as described herein, and a pharmaceutically acceptable excipient. In some embodiments, the composition includes a recombinant cell as

described herein, and a pharmaceutically acceptable excipient. In some embodiments, the composition is formulated with a delivery vehicle into a delivery system, wherein the delivery system includes a liposome, a viral replicon particle (VRP), a lipid-based nanoparticle (LNP), a polymer nanoparticle, a physiologic buffer, a microsphere, an immune stimulating complex (ISCOM), a conjugate of bioactive ligand, or a combination of any thereof. In some embodiments, the LNP delivery system includes a cationic lipid, an ionizable cationic lipid, an anionic lipid, and/or a neutral lipid. In some embodiments, the lipid of the LNP delivery system is present in mass ratio of lipid to RNA from about 100:1 to about 4:1. In some embodiments, the delivery system includes lipid-based nanoparticles having an average diameter of less than 1000 nm, less than 500 nm, less than 250 nm, less than 200 nm, less than 150 nm, less than 100 nm, less than 75 nm, less than 50 nm, or less than 25 nm. In some embodiments, the LNP delivery system includes lipid-based nanoparticles having an average diameter of about 1 - 1000 nm, about 1 - 1000 nm, about 1 - 500 nm, about 1 - 250 nm, about 25 - 200 nm, about 25 - 100 nm, about 35 - 75 nm, or about 25 - 60 nm.

[0020] In some embodiments, the pharmaceutical composition disclosed herein is an immunogenic composition. In some embodiments, the composition is formulated as a vaccine or an adjuvant. In some embodiments, the composition is formulated for one or more of the following administration routes: 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. In some particular embodiments, the composition is formulated intramuscular administration.

[0021] In another aspect, provided herein are methods for eliciting a pharmacodynamic effect in a subject in need thereof, the method includes administering to the subject a composition including: (a) a nucleic acid construct as described herein; (b) a recombinant cell as described herein; and/or (c) a pharmaceutical composition as described herein. Non-limiting exemplary embodiments of the methods for eliciting a pharmacodynamic effect disclosed herein can include one or more of the following features. In some embodiments, the pharmacodynamic effect includes 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. In some embodiments, the pharmacodynamic effect includes preventing infection and/or decreasing viral load in the subject. In some embodiments, the pharmacodynamic effect includes preventing and/or decreasing disease severity in the subject. In some embodiments, the pharmacodynamic effect includes eliciting an immune response in the subject. In some embodiments, the immune response includes one or more of the following: an anti-EBV antibody response, a neutralizing antibody response, and an anti-EBV T cell response.

[0022] In another aspect, provided herein are methods for preventing and/or treating a health condition in a subject in need thereof, the method includes prophylactically or therapeutically administering to the subject a composition including: (a) a nucleic acid construct as described herein; (b) a recombinant cell as described herein; and/or (c) a pharmaceutical composition as described herein. Non-limiting exemplary embodiments of the methods for preventing and/or treating a health condition disclosed herein can include one or more of the following features. In some embodiments, the administered composition elicits an immune response in the subject. In some embodiments, the administered composition results in an increased production of interferon in the subject. In some embodiments, the subject is having or suspected of having an EBV-associated disease. In some embodiments, the EBV-associated disease is infectious mononucleosis (IM), an autoimmune disease, or a cancer.

[0023] In some embodiments, the composition is administered to the subject individually as a single agent prophylaxis or therapy (monotherapy) or as a first therapy in combination with at least one additional therapies. In some embodiments, the at least one additional therapies is selected from the group consisting of chemotherapy, radiotherapy, immunotherapy, hormonal therapy, toxin therapy, targeted therapy, and surgery.

[0024] In yet another aspect, provided herein are kits for eliciting a pharmacodynamic effect, eliciting an immune response, and/or for the prevention and/or treatment of a health condition, the kit including: (a) a nucleic acid construct as described herein; (b) a recombinant cell as described herein; and/or (c) a pharmaceutical composition as described herein, and instructions for performing one or more methods of the present disclosure. In some embodiments, the health condition is an EBV-associated disease. In some embodiments, the EBV-associated disease is infectious mononucleosis (IM), an autoimmune disease, or a cancer.

[0025] Each of the aspects and embodiments described herein are capable of being used together, unless excluded either explicitly or clearly from the context of the embodiment or aspect.

[0026] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative embodiments and features described herein, further aspects, embodiments, objects and features of the disclosure will become fully apparent from the drawings and the detailed description and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] **FIG. 1** is graphical representation of a non-limiting example of a multigenic EBV vaccine design in accordance with some embodiments of the disclosure, in which the nucleic acid sequence encoding viral structural proteins of the original virus have been completely deleted. The multigenic EBV vaccine design described in this figure contains native 5' UTR and 3' UTR derived from an alphavirus, and further contains a heterologous gene of interest (GOI), which is a multigenic expression cassette placed under control of a 26S subgenomic promoter. The coding sequences for the non-structural proteins nsP1, nsP2, nsP3, and nsP4 are indicated.

[0028] **FIG. 2** shows schematic depictions of twenty-two (22) exemplary nucleic acid constructs expressing EBV antigenic determinants derived from one or more EBV polypeptides/antigens in accordance with some embodiments of the disclosure. In each construct, the numbers of EBV antigens and their ordinality in the antigen cassette are shown. While some constructs contain coding sequences for multiple polypeptides operably linked to one another within a single open reading frame (*e.g.*, in a polycistronic ORF), others include coding sequences for multiple polypeptides configured into two ORFs that are connected to one another by a sequence for an internal ribosomal entry site (IRES). P2A: autoproteolytic peptide sequence from porcine teschovirus-1 2A. All gp350- and gH- coding sequences described in FIG. 2, with the exception of gH in Rep-678, lack functional transmembrane domains (-TM). The coding sequences for BZLF1 described in FIG. 2 are devoid of (i) a functional nuclear localization sequence (NLS) and (ii) a functional transactivation (TA) domain. In this figure, the EBV polypeptides, autoproteolytic peptide P2A sequences, and internal ribosomal entry sites (IRES) are shown in N-terminus to C-terminus direction (*i.e.*, in 5' to 3' direction) of the corresponding coding sequences.

[0029] **FIG. 3** schematically summarizes the results of experiments performed to illustrate

srRNA-based bioactivity as measured by a replication intermediate dsRNA in BHK-21 cells electroporated with various EBV srRNA vaccines. In this figure, the frequency of dsRNA+ cells is plotted for each construct.

[0030] **FIG 4** schematically summarizes the results of experiments performed to illustrate protein expression from three out of five antigens in various multigenic or monogenic srRNA vaccines in electroporated BHK-21 cells. In these experiments, protein expression was detected by flow cytometry using antibodies to gp350 and gH/gL complex. Mean fluorescence intensity (MFI) of the fluorescent-labeled antibody is plotted as a measure of protein expression.

[0031] **FIG. 5** pictorially summarizes the results of experiments performed to illustrate protein expression for BZLF1 in various monogenic and multigenic srRNA vaccines in electroporated BHK-21 cells. In these experiments, protein expression was detected by Western blotting using whole cell lysates from electroporated BHK-21 cells using an antibody to BZLF1. Expression of actin was used as a loading control for the samples.

[0032] **FIGS. 6A-6B** schematically summarize the results of experiments performed to demonstrate that srRNA-based vaccines can generate antibody responses *in vivo* (ELISA) to encoded EBV antigens. In these experiments, antibody responses were measured 13 days after two doses of srRNA-based vaccines (1 µg each) by total IgG ELISA to three surface EBV antigens: gH/gL complex (**FIG. 6A**) and gp350 (**FIG. 6B**). EBV-binding IgG antibody responses were measured by a direct ELISA method to EBV proteins gp350 and gH/gL complex using sera from immunized mice. A standard mouse antibody to each EBV antigen was used to quantify responses.

[0033] **FIGS. 7A-7D** schematically summarize the results of experiments performed to demonstrate that srRNA-based vaccines can generate T cell responses *in vivo* (ELISpot) to encoded EBV antigens. In these experiments, T cell responses were measured 13 days after two doses of srRNA-based vaccines (1 µg) by IFN γ ELISpot for five encoded antigens: gH and gL (**FIG. 7A**), gp350 (**FIG. 7B**), LMP2B (**FIG. 7C**), and BZLF1 (**FIG. 7D**). T cell responses were measured by stimulation of whole splenocytes with peptide pools corresponding to each encoded antigen and detecting secretion of IFN γ using a commercial ELISpot kit.

[0034] **FIG. 8** schematically summarizes the results of experiments performed to illustrate that srRNA-based EBV vaccines can generate neutralizing antibody responses *in vivo* to EBV.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0035] Provided herein are, *inter alia*, viral expression systems including self-replicating RNAs (srRNAs) based on alphaviruses with superior expression potential which are suitable for expressing heterologous molecules such as, antigens and antigenic determinants from Epstein-Barr virus (EBV), for the purposes of therapeutic treatment of human health conditions or diseases, including posttransplant lymphoproliferative disease (PTLD), autoimmune diseases, inflammatory diseases, and cancers. These srRNA-based expression systems address the problem with the complexity of EBV lifecycle and infection. For example, some embodiments of the disclosure relate to nucleic acid constructs such as, *e.g.*, expression constructs and vectors, containing a modified genome or srRNA of an alphavirus in which at least a portion of the nucleic acid sequence encoding the viral structural proteins of the modified alphavirus genome or srRNA has been replaced by coding sequences for a polypeptide construct containing multiple antigenic determinants derived from EBV. Further provided are recombinant cells that are genetically engineered to include one or more of the nucleic acid constructs disclosed herein. Biomaterials and recombinant products derived from such recombinant cells are also within the scope of the application. Also provided are compositions and methods useful for (i) modulating a pharmacodynamic effect and for preventing and/or treating a health condition associated with EBV in a subject in need thereof.

DEFINITIONS

[0036] Unless otherwise defined, all terms of art, notations, and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this application pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art.

[0037] The singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes one or more cells, comprising mixtures thereof. “A and/or B” is used herein to include all of the following alternatives: “A,” “B,”

“A or B,” and “A and B.”

[0038] The terms “administration” and “administering,” as used herein, refer to the delivery of a bioactive composition or formulation by an administration route comprising, but not limited to, intranasal, transdermal, intravenous, intra-arterial, intramuscular, intranodal, intraperitoneal, subcutaneous, intramuscular, oral, intravaginal, and topical administration, or combinations thereof. The term includes, but is not limited to, administering by a medical professional and self-administering.

[0039] The terms “cell,” “cell culture,” and “cell line” refer not only to the particular subject cell, cell culture, or cell line but also to the progeny or potential progeny of such a cell, cell culture, or cell line, without regard to the number of transfers or passages in culture. It should be understood that not all progeny are exactly identical to the parental cell. This is because certain modifications may occur in succeeding generations due to either mutation (*e.g.*, deliberate or inadvertent mutations) or environmental influences (*e.g.*, methylation or other epigenetic modifications), such that progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein, so long as the progeny retain the same functionality as that of the original cell, cell culture, or cell line.

[0040] The term “antigenic determinant” refers to that portion (*e.g.*, fragment or site) of an antigen molecule that is specifically recognized by either B- or T-lymphocytes. An example of antigenic determinant is an epitope that interacts with an antigen-binding site of an antigen-binding polypeptide, *e.g.*, a variable region of an antibody molecule, or of a T cell receptor (TCR) when the antigen is presented on major histocompatibility complex (MHC). B-lymphocytes respond to foreign antigenic determinants via antibody production, whereas T-lymphocytes are the mediator of cellular immunity. Thus, antigenic determinants or epitopes are those parts of an antigen that are recognized by antibodies, or in the context of an MHC, by T-cell receptors. Epitopes can be linear or conformational, the latter may or may not be composed of continuous sequence of amino acids. In certain embodiments, epitopes can include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, can have specific three-dimensional structural characteristics, and/or specific charge characteristics. One skilled in the art will understand that a single antigen may have more than one antigenic determinant or epitope. Thus, different antibodies may bind to

different areas on an antigen and may have different biological effects. The term epitope also refers to a site on an antigen to which B and/or T cells respond. It also refers to a portion (*e.g.*, region, fragment, or site) of an antigen that is bound by an antibody or a TCR.

[0041] The term “effective amount,” “therapeutically effective amount,” or “pharmaceutically effective amount” of a composition of the disclosure, *e.g.*, nucleic acid constructs (*e.g.*, srRNA constructs), recombinant cells, and/or pharmaceutical compositions, generally refers to an amount sufficient for the composition to accomplish a stated purpose relative to the absence of the composition (*e.g.*, achieve the effect for which it is administered, stimulate an immune response, prevent or treat a disease, or reduce one or more symptoms of a disease, disorder, infection, or health condition). An example of an “effective amount” is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a “therapeutically effective amount.” A “reduction” of a symptom means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). The exact amount of a composition including a “therapeutically effective amount” will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, *e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and Remington: *The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0042] The term “construct” refers to a recombinant molecule, *e.g.*, recombinant nucleic acid or polypeptide, including one or more isolated nucleic acid sequences or amino acid sequences from heterologous sources. For example, polypeptide constructs can be chimeric polypeptide molecules in which two or more amino acid sequences of different origin are operably linked to one another in a single polypeptide construct. Similarly, nucleic acid constructs can be chimeric nucleic acid molecules in which two or more nucleic acid sequences of different origin are assembled into a single nucleic acid molecule. Thus, representative nucleic acid constructs include any constructs that contain (1) nucleic acid sequences, including regulatory and coding sequences that are not found adjoined to one another in nature (*e.g.*, at least one of the nucleotide sequences is heterologous with respect to at least one of its other nucleotide sequences), or (2) sequences encoding parts of functional RNA molecules or proteins not naturally adjoined, or (3) parts of

promoters that are not naturally adjoined. Representative nucleic acid constructs can include any recombinant nucleic acid molecules, linear or circular, single-stranded or double-stranded DNA or RNA nucleic acid molecules, derived from any source, such as a plasmid, cosmid, virus, autonomously replicating polynucleotide molecule, phage, capable of genomic integration or autonomous replication, comprising a nucleic acid molecule where one or more nucleic acid sequences have been operably linked. In some embodiments, nucleic acid constructs of the present disclosure can include the necessary elements to direct expression of a nucleic acid sequence of interest that is also contained in the construct. Such elements may include control elements such as a promoter that is operably linked to (*e.g.*, so as to direct transcription of) the nucleic acid sequence of interest, and optionally includes a polyadenylation sequence.

[0043] In some embodiments of the disclosure, the nucleic acid construct may be incorporated within a vector. The term “vector” is used herein to refer to a nucleic acid molecule or sequence capable of transferring or transporting another nucleic acid molecule. Thus, the term “vector” encompasses both DNA-based vectors and RNA-based vectors. The term “vector” includes cloning vectors and expression vectors, as well as viral vectors and integrating vectors. An “expression vector” is a vector that includes a regulatory region, thereby capable of expressing DNA sequences and fragments *in intro*, *ex vivo*, and/or *in vivo*. In some embodiments, a vector may include sequences that direct autonomous replication in a cell such as, for example a plasmid (DNA-based vector) or a self-replicating RNA vector. In some embodiments, a vector may include sequences sufficient to allow integration into host cell DNA. Useful vectors include, for example, plasmids (*e.g.*, DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. In some embodiments, the vector of the disclosure can be single-stranded vector (*e.g.*, ssDNA or ssRNA). In some embodiments, the vector of the disclosure can be double-stranded vector (*e.g.*, dsDNA or dsRNA). In some embodiments, a vector is a gene delivery vector. In some embodiments, a vector is used as a gene delivery vehicle to transfer a gene into a cell.

[0044] In addition to the components of the construct, the vector may include, for example, one or more selectable markers, one or more origins of replication, such as prokaryotic and eukaryotic origins, at least one multiple cloning site, and/or elements to facilitate stable integration of the construct into the genome of a cell. Two or more constructs can be incorporated within a

single nucleic acid molecule, such as a single vector, or can be incorporated within two or more separate nucleic acid molecules, such as two or more separate vectors. An “expression construct” generally includes at least a control sequence operably linked to a nucleotide sequence of interest. In this manner, for example, promoters in operable connection with the nucleotide sequences to be expressed are provided in expression constructs for expression in a cell. For the practice of the present disclosure, compositions and methods for preparing and using constructs and cells are known to one skilled in the art.

[0045] The term “operably linked,” as used herein, denotes a physical or functional linkage between two or more elements, *e.g.*, polypeptide sequences or polynucleotide sequences, which permits them to operate in their intended fashion. For example, the term “operably linked” when used in context of the nucleic acid molecules described herein or the coding sequences and promoter sequences in a nucleic acid molecule means that the coding sequences and promoter sequences are in-frame and in proper spatial and distance away to permit the effects of the respective binding by transcription factors or RNA polymerase on transcription. It should be understood that operably linked elements may be contiguous or non-contiguous (*e.g.*, linked to one another through a linker). Thus, operably linked segments, portions, regions, and domains of the polypeptides or nucleic acid molecules disclosed herein may be contiguous or non-contiguous (*e.g.*, linked to one another through a linker). In the context of polypeptide constructs, “operably linked” refers to a physical linkage (*e.g.*, directly or indirectly linked) between amino acid sequences (*e.g.*, different segments, portions, regions, or domains) to provide for a described activity of the constructs.

[0046] The term “portion” as used herein refers to a fraction. With respect to a particular structure such as a polynucleotide sequence or an amino acid sequence or protein the term “portion” thereof may designate a continuous or a discontinuous fraction of said structure. For example, a portion of an amino acid sequence comprises at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, and at least 90% of the amino acids of said amino acid sequence. In addition or alternatively, if the portion is a discontinuous fraction, said discontinuous fraction is composed of 2, 3, 4, 5, 6, 7, 8, or more parts of a structure (*e.g.*, domains of a protein), each part being a continuous element of the structure. For example, a discontinuous fraction of an amino acid sequence may be composed of 2,

3, 4, 5, 6, 7, 8, or more, for example not more than 4 parts of said amino acid sequence, wherein each part comprises at least 1, at least 2, at least 3, at least 4, at least 5 continuous amino acids, at least 10 continuous amino acids, at least 20 continuous amino acids, or at least 30 continuous amino acids of the amino acid sequence.

[0047] The term “recombinant” when used with reference to a cell, a nucleic acid, a protein, or a vector, indicates that the cell, nucleic acid, protein or vector has been altered or produced through human intervention such as, for example, has been modified by or is the result of laboratory methods. Thus, for example, recombinant proteins and nucleic acids include proteins and nucleic acids produced by laboratory methods. Recombinant proteins can include amino acid residues not found within the native (non-recombinant or wild-type) form of the protein or can be include amino acid residues that have been modified, *e.g.*, labeled. The term can include any modifications to the peptide, protein, or nucleic acid sequence. Such modifications may include the following: any chemical modifications of the peptide, protein or nucleic acid sequence, including of one or more amino acids, deoxyribonucleotides, or ribonucleotides; addition, deletion, and/or substitution of one or more of amino acids in the peptide or protein; creation of a fusion protein, *e.g.*, a fusion protein comprising an antibody fragment; and addition, deletion, and/or substitution of one or more of nucleic acids in the nucleic acid sequence. The term “recombinant” when used in reference to a cell is not intended to include naturally-occurring cells but encompass cells that have been engineered/modified to include or express a polypeptide or nucleic acid that would not be present in the cell if it was not engineered/modified.

[0048] The term “percent sequence identity,” as used herein in the context of two or more nucleic acids or proteins, refers to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acids that are the same (*e.g.*, about 60% sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection. See *e.g.*, the NCBI website at ncbi.nlm.nih.gov/BLAST. Such sequences are then said to be “substantially identical.” This definition also refers to, or may be applied to, the complement of a query sequence. This definition includes sequence comparison

performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences. This definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. Sequence identity can be calculated over a region that is at least about 20 amino acids or nucleotides in length, or over a region that is 10-100 amino acids or nucleotides in length, or over the entire length of a given sequence. Sequence identity can be calculated using published techniques and widely available computer programs, such as the GCS program package (Devereux *et al.*, Nucleic Acids Res (1984) 12:387), BLASTP, BLASTN, FASTA (Atschul *et al.*, J Mol Biol (1990) 215:403). Sequence identity can be measured using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group at the University of Wisconsin Biotechnology Center (1710 University Avenue, Madison, Wis. 53705), with the default parameters thereof. Additional methodologies that can suitably be utilized to determine similarity or identity amino acid sequences include those relying on position-specific structure-scoring matrix (P3SM) that incorporates structure-prediction scores from Rosetta, as well as those based on a length-normalized edit distance as described previously in, *e.g.*, Setcliff *et al.*, Cell Host & Microbe 23(6), May 2018.

[0049] The term “pharmaceutically acceptable excipient” as used herein refers to any suitable substance that provides a pharmaceutically acceptable carrier, additive, or diluent for administration of a compound(s) of interest to a subject. As such, “pharmaceutically acceptable excipient” can encompass substances referred to as pharmaceutically acceptable diluents, pharmaceutically acceptable additives, and pharmaceutically acceptable carriers. As used herein, the term “pharmaceutically acceptable carrier” includes, but is not limited to, saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds (*e.g.*, antibiotics and additional therapeutic agents) can also be incorporated into the compositions.

[0050] As used herein, a “subject” or an “individual” includes animals, such as human (*e.g.*, human individuals) and non-human animals. In some embodiments, a “subject” or “individual” is a patient under the care of a physician. Thus, the subject can be a human patient or an individual who has, is at risk of having, or is suspected of having a health condition of interest (*e.g.*, EBV-

associated cancer or infection) and/or one or more symptoms of the health condition. The subject can also be an individual who is diagnosed with a risk of the health condition of interest at the time of diagnosis or later. The term “non-human animals” includes all vertebrates, *e.g.*, mammals, *e.g.*, rodents, *e.g.*, mice, non-human primates, and other mammals, such as *e.g.*, sheep, dogs, cats, cows, chickens, and non-mammals, such as amphibians, reptiles, *etc.*

[0051] It is understood that aspects and embodiments of the disclosure described herein include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments. As used herein, “comprising” is synonymous with “including,” “containing,” or “characterized by,” and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, “consisting of” excludes any elements, steps, or ingredients not specified in the claimed composition or method. As used herein, “consisting essentially of” does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claimed composition or method. Any recitation herein of the term “comprising,” particularly in a description of components of a composition or in a description of steps of a method, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or steps.

[0052] Where a range of values is provided, it is understood by one having ordinary skill in the art that all ranges disclosed herein encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* In some embodiments, where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure. As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like include the

number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Furthermore, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0053] Certain ranges are presented herein with numerical values being preceded by the term “about” which, as used herein, has its ordinary meaning of approximate. The term “about” is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number. If the degree of approximation is not otherwise clear from the context, “about” means either within plus or minus 10% of the provided value, or rounded to the nearest significant figure, in all cases inclusive of the provided value. In some embodiments, the term “about” indicates the designated value $\pm$ up to 10%, up to $\pm$ 5%, or up to $\pm$ 1%.

[0054] Headings, *e.g.*, (a), (b), (i) *etc.*, are presented merely for ease of reading the specification and claims. The use of headings in the specification or claims does not require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.

[0055] It should be appreciated that certain features of the disclosure, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosure, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the disclosure are specifically embraced by the present disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present disclosure and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

EPSTEIN-BARR VIRUS

[0056] Epstein-Barr virus (EBV), also referred to as human herpesvirus 4 (HHV-4), is a common human herpesvirus, which belongs to the gamma-family of viruses along with one other human virus, Kaposi's sarcoma associated herpesvirus (KHSV). Unlike alpha- and beta-herpesviruses, these viruses share the ability to induce cancer. EBV belongs to the gamma 1 or lymphocryptovirus genus and was identified as the first human tumor virus. EBV can be divided into two major types, EBV type 1 (EBV-1; also referred to as EBV type A) and EBV type 2 (EBV-2 or EBV type B). These two subtypes have different EBNA-3 genes. As a result, the two subtypes differ in their transforming capabilities and reactivation ability. Type 1 is dominant throughout most of the world, but the two types are equally prevalent in Africa.

[0057] EBV is associated with a wide variety of diseases and malignancies. For example, EBV is the cause of infectious mononucleosis (glandular fever) and associated with particular forms of proliferative diseases such as posttransplant lymphoproliferative disease (PTLD), autoimmune diseases, inflammatory diseases and cancers, *e.g.*, Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, as well as health conditions associated with human immunodeficiency virus (HIV), *e.g.*, hairy leukoplakia and central nervous system lymphomas.

[0058] In particular, EBV infection is associated with a higher risk of certain autoimmune diseases, especially dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, and multiple sclerosis. EBV infects more than 90% of the global population, but it exists in a latent state in most infected individuals and escapes immune surveillance. Each year approximately 20,000 cancer cases are attributed to EBV, such as B-cell malignancies, nasopharyngeal cancer (NPC), gastric cancer (GC), and some rare T/NK cell lymphomas, leukemias, and leiomyosarcomas. The epithelial cancers NPC and GC are the major group of EBV-associated malignancies with high mortalities, of which EBV+GC is the largest category of EBV-positive cancer, with more than 80,000 cases per year.

[0059] Infectious mononucleosis (glandular fever) is an extremely common, self-limiting, and acute disease associated with primary EBV infection. It is characterized by lymphadenopathy, transient fever and hepatosplenomegaly that usually resolves in time. Chronic active EBV infection (CAEBV), although rare, is a severe and fatal condition characterized by unusually high EBV DNA load (103–107 copies/mL), which is now considered to be one of the EBV+ T or NK

cell lymphoproliferative diseases and can lead to two lethal conditions: hemophagocytic lymphohistiocytosis (HLH) and chemotherapy-resistant lymphoma.

EBV Life cycle and infection

[0060] Epstein-Barr Virus (EBV) exhibits a biphasic lifecycle that includes latent and lytic (replicative) phases. The typical transmission route is through bodily fluids, such as saliva, where the orally transmitted virions infect resting B and epithelial cells of the oral cavity. Primary infection is typically asymptomatic, although 35-50% of the human adolescent population develop infectious mononucleosis (IM) approximately 1 month after infection, and the virus persists throughout an individual's life. After acute infection, a dormant state is established due to a strong, virus-specific T cell response. However, when the balance between the virus and host immune system is disrupted, EBV can drive malignant transformation of both lymphoid and epithelial origins, causing ~200,000 deaths annually.

[0061] As a herpesvirus, EBV can cause either latent or lytic infection. In epithelial cells, EBV typically undergoes lytic replication. In B cells, EBV usually establishes lifelong latency with rare sporadic reactivations. During latency only a few essential viral genes are expressed and production of virions is stalled. The switch from latent to lytic phase is governed by several factors. While EBV-encoded products in both phases can play a role in transformation and tumorigenesis, the literature is more extensive on the oncogenic role of latent genes compared to lytic genes. However, it is challenging to target latent EBV using current immunotherapeutic strategies, specifically due to reduced antigen expression. As a result, patients with EBV+ or EBV- tumors are typically subjected to similar treatment regimens.

[0062] Upon infection, the virus typically establishes latency within the host cell. During latency, only a handful of latent genes that are necessary for the maintenance and persistence of the viral genome are expressed. EBV encodes eight latency genes whose expression in host cells and/or malignancies defines EBV latency programs. Based on which of the eight latent viral genes are expressed, viral infection is categorized into three main latency programs, latency III, II and I/0. EBV-infected naïve B cells exhibit a latency III program, which allows for the proliferation and expansion of infected cells. Latency III genes include 6 EBV nuclear antigens (EBNA1, 2, 3A, 3B, 3C, LP), 2 latent membrane proteins (LMP1 and LMP2), EBV-encoded small RNAs (EBERs), and EBV-encoded microRNAs (miRNAs). The LMP2 gene encodes for 2 protein isoforms

LMP2A and LMP2B. LMP2B is missing exon 1 but otherwise overlaps with LMP2A. The amino acid sequence of LMP2B lacks exon 1 but otherwise overlaps with LMP2A.

[0063] The cells in this latency program are highly immunogenic and can be rapidly eliminated by the host immune response, specifically by EBV-specific T cells. Latency II has a more restricted expression of EBV genes, namely EBNA1, LMP1, and LMP2A/B making them less immunogenic. Eventually, EBV sequentially shuts down the expression of all the latent genes except EBNA1 and a few EBV-encoded RNAs in latency I. Latency II can also be divided to IIa and IIb based on the expression of LMPs and EBNA2-3 (IIb is EBNA2-3+LMP-; IIa is EBNA2-3-LMP+). In most individuals, EBV persists quiescently within a subset of memory B cells (<0.005% B cells in the peripheral blood) without expressing any viral genes in latency 0 state, also referred to as a “true latency.” Latent EBV genes are reported to promote tumorigenesis, inhibit apoptosis, and suppress recognition of infected cells by host immune cells. EBV-related malignancies are linked with different EBV latency programs. Lymphoproliferative disorders that are commonly associated with immunosuppression such as post-transplant lymphoproliferative diseases (PTLDs) and acquired immunodeficiency syndrome (AIDs) associated lymphomas exhibit latency III. Hodgkin lymphoma, T/NK cell lymphomas and nasopharyngeal carcinoma (NPC) exhibit latency II. Gastric carcinoma and Burkitt lymphoma exhibit latency I program (27, 31). So far, EBV in latency 0 has not been associated with any malignancies, presumably due to dormancy during this program.

[0064] The lytic phase is necessary for EBV progeny production and horizontal transmission of virus from host to host, so represents an integral aspect of viral pathogenesis (32). The switch from latent to lytic cycle can be either spontaneous or chemically induced. Some of the commonly used agents to induce lytic cycle include phorbol esters (PMA), sodium butyrate, calcium ionophores, DNA methyltransferase inhibitors (DNMTi), transforming growth factor-beta (TGF- β), doxorubicin and gemcitabine (because these are stress inducing chemotherapeutic drugs) and anti-IgG or anti-IgM as B-cell receptor stimulants. During lytic reactivation, the full repertoire of >80 viral genes is temporally regulated and expressed during three phases - immediate early (IE), early (E), and late (L). The first phase is primarily initiated by BZLF1 (ZEBRA) and BRLF1, the two key EBV immediate-early (IE) lytic transcription factors. Both genes function to promote their own and each other's expression, as well as the expression of viral E genes, that code for proteins

needed for viral replication (*e.g.*, viral DNA polymerase). BZLF1 forms a homodimer via its basic leucine zipper motif and binds to BZLF1-responsive elements (ZRE) on DNA. The binding of BZLF1 to CpG methylated DNA leads to activation of several lytic viral genes that are silenced in latent cells by CpG methylation. In addition, binding of BZLF1 to the origin of lytic replication (*oriLyt*) ZRE promotes lytic viral DNA synthesis. Similarly, BRLF1 binds to the BRLF1-responsive elements (RRE) on DNA and is reported to induce lytic replication via the PI3K and ERK signaling pathways. Both BZLF1 and BRLF1 are quintessential for EBV lytic replication since knocking out these genes blocks the latent to lytic switch. In addition, overexpression of BZLF1 and BRLF1 in latently infected cells can induce EBV lytic reactivation. This lytic induction leads to a cascade of viral gene expression, which promote viral DNA replication and virion production. Following viral replication, late viral genes code for structural proteins, such as gp350/220 encoded by the BLLF1 gene are expressed. Interestingly, during lytic DNA replication in γ -herpesviruses, continuous DNA synthesis is needed for the transcription of late lytic viral genes but not for early lytic genes. The virions can disseminate viral particles within host cells and among hosts. EBV replicates in latency I, II and III via proliferation of activated B cells. It has been reported that lytic replication can only be efficiently induced from latency I/0, and after extensive methylation of the viral genome. This is because BZLF1 prefers binding to methylated CpG sequences to initiate infectious particle production.

[0065] Currently there are no FDA approved vaccines to prevent primary EBV infection and/or EBV-associated diseases on the market. Therefore, there is a pressing need in the art for developing methods and agents, *e.g.*, prophylactic vaccines and therapeutic vaccines against EBV and EBV-associated diseases, such as infectious mononucleosis and EBV-positive cancers.

ALPHAVIRUS SELF-REPLICATING RNAS

Alphaviruses

[0066] In some embodiments of the disclosure, the replicon, *e.g.*, srRNA, is derived from a virus belonging to the *Alphavirus* genus. The *Alphavirus* genus has been widely studied and the life cycle, mode of replication, *etc.*, of these viruses are well characterized. Alphaviruses are small, enveloped RNA viruses with a single-stranded, positive-sense RNA genome. More information in this regard can be found in, *e.g.*, Arrigo NC *et al.*, supra 2010 and Corrin T. *et al.*, Vector-Borne and Zoonotic Diseases, Vol. 21, No. 5, 2021. In addition, alphaviruses have been shown to

replicate very efficiently in animal cells which makes them valuable as vectors for production of protein and nucleic acids in such cells. Transmission between species and individuals occurs mainly via mosquitoes making the alphaviruses a contributor to the collection of Arboviruses, also known as Arthropod-Borne Viruses.

[0067] The *Alphavirus* genus includes, *inter alia*, the Sindbis virus (SINV), the Semliki Forest virus (SFV), the Ross River virus (RRV), Venezuelan equine encephalitis virus (VEEV), and Eastern equine encephalitis virus (EEEV), which are all closely related and are able to infect various vertebrates such as mammals, rodents, fish, avian species, and larger mammals such as humans and horses as well as invertebrates such as insects. Each of these alphaviruses has a single stranded RNA genome of positive polarity enclosed in a nucleocapsid surrounded by an envelope containing viral spike proteins. *Alphavirus* particles are enveloped, tend to be spherical (although slightly pleomorphic), and have an isometric nucleocapsid. *Alphavirus* genomes are single-stranded RNA of positive polarity of approximately 11-12 kb in length, flanked by 5' and 3' untranslated regions (UTRs) and are capped at the 5' end and polyadenylated at the 3' end, and comprise two open reading frames with a first frame (~7 kb) encoding the nonstructural proteins with enzymatic function and a second frame (~4 kb) encoding the viral structural proteins (*e.g.*, the capsid protein CP, E1 glycoprotein, E2 glycoprotein, E3 protein and 6K protein). The non-structural polyprotein (nsP) is cleaved into four different proteins (nsP1, nsP2, nsP3, and nsP4) which are necessary for the transcription and translation of viral mRNA inside the cytoplasm of host cells.

[0068] The 5' two-thirds of the alphavirus genome encodes a number of nonstructural proteins (nsPs) necessary for transcription and replication of viral RNA. These proteins are translated directly from the RNA and together with cellular proteins form the RNA-dependent RNA polymerase essential for viral genome replication and transcription of subgenomic RNA. Four nonstructural proteins (nsP1, nsP2, nsP3, nsP4) are produced as a single polyprotein constitute the virus' replication machinery. The processing of the polyprotein occurs in a highly regulated manner, with cleavage at the P2/3 junction influencing RNA template use during genome replication. This site is located at the base of a narrow cleft and is not readily accessible. Once cleaved, nsP3 creates a ring structure that encircles nsP2. These two proteins have an extensive interface. Mutations in nsP2 that produce noncytopathic viruses or a temperature

sensitive phenotypes cluster at the P2/P3 interface region. P3 mutations opposite the location of the nsP2 noncytopathic mutations prevent efficient cleavage of P2/3. This in turn can affect RNA infectivity altering viral RNA production levels.

[0069] The 3' one-third of the genome comprises subgenomic RNA which serves as a template for translation of all the structural proteins required for forming viral particles: the core nucleocapsid protein C, and the envelope proteins P62 and E1 that associate as a heterodimer. The viral membrane-anchored surface glycoproteins are responsible for receptor recognition and entry into target cells through membrane fusion. The subgenomic RNA is transcribed from the p26S subgenomic promoter present at the 3' end of the RNA sequence encoding the nsP4 protein. The proteolytic maturation of P62 into E2 and E3 causes a change in the viral surface. Together the E1, E2, and sometimes E3, glycoprotein "spikes" form an E1/E2 dimer or an E1/E2/E3 trimer, where E2 extends from the center to the vertices, E1 fills the space between the vertices, and E3, if present, is at the distal end of the spike. Upon exposure of the virus to the acidity of the endosome, E1 dissociates from E2 to form an E1 homotrimer, which is necessary for the fusion step to drive the cellular and viral membranes together. The alphaviral glycoprotein E1 is a class II viral fusion protein, which is structurally different from the class I fusion proteins found in influenza virus and HIV. The E2 glycoprotein functions to interact with the nucleocapsid through its cytoplasmic domain, while its ectodomain is responsible for binding a cellular receptor. Most alphaviruses lose the peripheral protein E3, while in Semliki viruses it remains associated with the viral surface.

[0070] Alphavirus replication has been reported to take place on membranous surfaces within the host cell. In the first step of the infectious cycle, the 5' end of the genomic RNA is translated into a polyprotein (nsP1-4) with RNA polymerase activity that produces a negative strand complementary to the genomic RNA. In a second step, the negative strand is used as a template for the production of two RNAs, respectively: (1) a positive genomic RNA corresponding to the genome of the secondary viruses producing, by translation, other nsP and acting as a genome for the virus; and (2) subgenomic RNA encoding the structural proteins of the virus forming the infectious particles. The positive genomic RNA/subgenomic RNA ratio is regulated by proteolytic autocleavage of the polyprotein to nsP1, nsP2, nsP3 and nsP4. In practice, the viral gene expression takes place in two phases. In a first phase, there is main synthesis of positive genomic strands and of negative strands. During the second phase, the synthesis of subgenomic RNA is

virtually exclusive, thus resulting in the production of large amount of structural protein.

Self-replicating RNA

[0071] As will be appreciated by the skilled artisan, the term “self-replicating RNA” (srRNA) refers to RNA molecule that contains all of the genetic information required for directing its own amplification or self-replication within a permissive cell. Therefore, srRNA is sometimes also referred to as “self-amplifying RNA” (saRNA). In some embodiments, the srRNA is a “replicon,” which can be a linear or circular section of DNA or RNA which replicates sequentially as a unit. Non-limiting examples of replicons include “replicon RNA” or “RNA replicon.” To direct its own replication, the srRNA generally (1) encodes polymerase, replicase, or other proteins which may interact with viral or host cell-derived proteins, nucleic acids or ribonucleoproteins to catalyze the RNA amplification process; and (2) contain *cis*-acting RNA sequences required for replication and transcription of the subgenomic RNA. These sequences may be bound during the process of replication to its self-encoded proteins, or non-self-encoded cell-derived proteins, nucleic acids or ribonucleoproteins, or complexes between any of these components.

[0072] In some embodiments of the disclosure, an alphavirus srRNA construct (*e.g.*, srRNA, saRNA, or RNA replicon molecule) generally contains the following elements: 5' viral or defective-interfering RNA sequence(s) required in *cis* for replication, sequences coding for biologically active alphavirus non-structural proteins (*e.g.*, nsP1, nsP2, nsP3, and nsP4), a subgenomic promoter (sg) for the subgenomic RNA (sgRNA), 3' viral sequences required in *cis* for replication, and optionally a polyadenylate tract (poly(A)). In some instances, a subgenomic promoter (sg) that directs expression of a heterologous sequence can be included in the srRNA construct of the disclosure.

[0073] Further, the term srRNA molecule (*e.g.*, srRNA, saRNA, or RNA replicon molecule) generally refers to a molecule of positive polarity, or “message” sense, and the srRNA may be of length different from that of any known, naturally-occurring alphavirus. In some embodiments of the present disclosure, the srRNA does not contain at least a portion of the coding sequence for one or more of the alphavirus structural proteins; and/or sequences encoding structural genes can be substituted with heterologous sequences. In those instances, where the srRNA is to be packaged into a recombinant alphavirus particle, it can contain one or more sequences, so-called packaging signals, which serve to initiate interactions with alphavirus structural proteins that lead to particle

formation.

[0074] The srRNA constructs of the disclosure generally have a length of at least about 2 kb. For example, the srRNA can have a length of at least about 2 kb, at least about 3 kb, at least about 4 kb, at least about 5 kb, at least about 6 kb, at least about 7 kb, at least about 8 kb, at least about 9 kb, at least about 10 kb, at least about 11 kb, at least about 12 kb or more than 12 kb. In some embodiments, the srRNA can have a length of about 4 kb to about 20 kb, about 4 kb to about 18 kb, about 5 kb to about 16 kb, about 6 kb to about 14 kb, about 7 kb to about 12 kb, about 8 kb to about 16 kb, about 9 kb to about 14 kb, about 10 kb to about 18 kb, about 11 kb to about 16 kb, about 5 kb to about 18 kb, about 6 kb to about 20 kb, about 5 kb to about 10 kb, about 5 kb to about 8 kb, about 5 kb to about 7 kb, about 5 kb to about 6 kb, about 6 kb to about 12 kb, about 6 kb to about 11 kb, about 6 kb to about 10 kb, about 6 kb to about 9 kb, about 6 kb to about 8 kb, about 6 kb to about 7 kb, about 7 kb to about 11 kb, about 7 kb to about 10 kb, about 7 kb to about 9 kb, about 7 kb to about 8 kb, about 8 kb to about 11 kb, about 8 kb to about 10 kb, about 8 kb to about 9 kb, about 9 kb to about 11 kb, about 9 kb to about 10 kb, or about 10 kb to about 11 kb. In some embodiments, the srRNA can have a length of about 6 kb to about 14 kb. In some embodiments, the srRNA can have a length of about 6 kb to about 16 kb.

COMPOSITIONS OF THE DISCLOSURE

[0075] As described in greater detail below, one aspect of the present disclosure relates to nucleic acid constructs a nucleic acid sequence encoding a modified viral genome or srRNA, wherein the modified genome or srRNA is devoid of (*e.g.* does not include) at least a portion of the nucleic acid sequence encoding one or more structural proteins of the corresponding unmodified viral genome or srRNA. Some embodiments of the disclosure provide a modified alphavirus genome or srRNA in which the coding sequence for non-structural proteins nsP1, nsP2, nsP3, and nsP4 is present, however at least a portion of or the entire sequence encoding one or more structural proteins is absent. Also provided are recombinant cells, cell cultures, and transgenic animals that have been engineered to include a nucleic acid construct as disclosed herein. The present disclosure further provides compositions, *e.g.*, pharmaceutical compositions, including one or more of the following: (a) a nucleic acid construct as described herein; and (b) a recombinant cell as described herein.

A. Nucleic acid constructs

[0076] As described in greater detail below, one aspect of the present disclosure relates to novel nucleic acid constructs encoding an alphavirus genome or an srRNA as described herein. In some embodiments, the nucleic acid constructs include a nucleic acid sequence encoding a modified genome or srRNA of an alphavirus, wherein at least a portion of the nucleic acid sequence encoding the viral structural proteins of the modified alphavirus genome or srRNA has been replaced by a coding sequence for a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV). For example, in some embodiments, a modified alphavirus genome or srRNA of the disclosure can include deletion(s), substitution(s), and/or insertion(s) in one or more of the genomic regions of the parent alphavirus genome. In some embodiments, the sequence encoding a srRNA of the disclosure can be operably linked, *e.g.*, placed under the control of elements required for expression (*e.g.*, promoter sequences), which allow expression of the srRNA in a host cell, in a subject, or in an *ex-vivo* cell-free expression system.

[0077] Non-limiting exemplary embodiments of the nucleic acid constructs, *e.g.*, genomes and srRNA constructs, of the disclosure can include one or more of the following features. In some embodiments, the one or more of antigenic determinants is derived from one or more EBV polypeptides/antigens encoded by a lytic gene, a pre-latent gene, a latent gene, an early gene, an immediate-early gene, a late gene, a reactivation from latency gene, a variant of any thereof, or a combination of any thereof. For example, in some embodiments, the polypeptide construct includes antigenic determinants derived from one or more EBV latent genes. Examples of EBV latent genes suitable for the compositions and methods of the present disclosure include BKRF1, BYRF1, BLRF3/BERF1, BERF2a/b, BERF3/4, BamHI-W, BNLF1 (LMP1), LMP2, BARTs, and EBER1/2. In some embodiments, the polypeptide construct includes antigenic determinants derived from the EBV latent/pre-latent gene LMP2, which is a transmembrane protein that is often expressed in latently infected cells. There are two isoforms of LMP2 (LMP2A and LMP2B). LMP2A is implicated in maintaining EBV latency. For example, LMP2A can exclude B-cell receptor (BCR) from lipid rafts to prevent lytic induction. The N-terminal region of LMP2A contains an immunoreceptor tyrosine-based activation motif (ITAM) that can lead to aberrant cell signaling in infected cells. In some embodiments, the polypeptide construct includes antigenic determinants derived from LMP2B isoform, which generally lacks 119 amino-terminal amino

acids compared to LMP2A including the ITAM. Without being bound to any particular theory or mechanism of action, the selection of LMP2B as a vaccine antigen in some embodiments of the present disclosure is believed to pose less of a safety risk than LMP2A while including antigenic determinants that overlap with the remaining homologous sequence between LMP2A and LMP2B.

[0078] In some embodiments, the polypeptide construct includes antigenic determinants derived from one or more EBV immediate-early genes. Non-limiting examples of immediate-early genes include BZLF1, BRLF1, and BMLF1. In some embodiments, the polypeptide construct includes one or more antigenic determinants derived from the EBV early immediate-gene BZLF1. In some embodiments, the polypeptide construct includes antigenic determinants derived from one or more EBV early genes. Suitable EBV early genes include, but are not limited to, BRRF1, BORF2, BaRF1, BXLF1, BGLF5, BLLF3, BKRF3, BALF5, BMRF1, BALF2, BSLF1, BBLF2/3, and BBLF4. Additional examples of EBV early genes suitable for the compositions and methods disclosed herein include BSLF2, BHRF1, BALF1, BARF1, BGLF4, BFRF1, BHLF1, BHLF2, and BNLF2a.

[0079] In some embodiments, the polypeptide construct includes antigenic determinants derived from one or more EBV late genes. Non-limiting examples of EBV late genes suitable for the compositions and methods of the present disclosure include BNRF1, BPLF1, BOLF1, BVRF1, BBLF1, BGLF1, BSRF1, BRRF2, BDLF2, BKRF4, BcLF1, BDLF1, BFRF3, BLRF2, BdRF1, BBRF1, BVRF2, BGLF2, BORF1, BLRF1 (gN). Additional examples of EBV late genes suitable for the compositions and methods disclosed herein include BLLF1 (gp350/220), BZLF2, BKRF2 (gp25, gL), BBRF3 (gM), BXLF2 (gp85, gH), BILF1, BILF2, BALF4 (gp100, gB), BDLF3, BMRF2, BALF3, and BCRF1.

[0080] In some embodiments, the polypeptide construct includes antigenic determinants derived from one or more EBV lytic genes. Suitable EBV early genes include, but are not limited to, BLLF1 (gp350/220), BXLF2 (gp85, gH), BALF4 (gp100, gB), BSLF1 (gp42), and BKRF2 (gp25, gL). One skilled in the art will appreciate that BALF4 (gp100, gB), BLLF1 (gp350/220), and BKRF2 (gp25, gL) can also be categorized as late genes. BSLF1 can also be categorized as an early gene.

[0081] In some embodiments, the polypeptide construct includes antigenic determinants derived from one or more EBV pre-latent gene genes. Examples of EBV pre-latent genes suitable

for the compositions and methods of the present disclosure include, but are not limited to, BNLF1 (LMP1), LMP2, BKRF1 (EBNA1), BLRF3/BERF1 (EBNA3A-C, -LP), and BYRF1 (EBNA2).

[0082] In some embodiments, the polypeptide construct includes one or more antigenic determinants derived from the EBV gene BLLF1, which is a glycoprotein important for efficient EBV infection of resting B cells. EBV open reading frame BLLF1 encodes the major envelope glycoproteins gp350 and gp220. A full-length open reading frame of this transcript encodes gp350, while a single splice of the primary transcripts deletes 197 codons and joins gp350 codons 401 and 699 in frame to generate gp220. Gp350 is the most abundant viral protein in the viral envelope. This large protein is heavily glycosylated and localizes to various subcellular compartments (cytoplasm, endoplasmic reticulum, Golgi, and plasma membrane) of replicating cells. EBV binds to primary B cells through its interaction with CD21, the complement receptor 2 (CR2) via gp350. Several gp350 domains have been reported to be involved in the formation of a stable complex with CD21, one of which has been identified as the receptor-binding site. This glycan-free domain is also recognized by the neutralizing gp350-specific antibody 72A. In some embodiments of the compositions and methods disclosed herein, the polypeptide construct includes one or more antigenic determinants derived from gp220. In some embodiments of the compositions and methods disclosed herein, the polypeptide construct includes one or more antigenic determinants derived from gp350. In some embodiments of the compositions and methods disclosed herein, the polypeptide construct includes one or more antigenic determinants derived only from gp350 and not from gp220.

[0083] In some embodiments, the polypeptide construct includes antigenic determinants derived from the EBV gH-gL complex which includes three glycoproteins, gp85, the gH homolog, and is the product of BXLF2; gp25, the gL homolog, which is the product of BKRF2; and gp42, which is the product of the BZLF2. The complex behaves in many respects like its counterparts in other herpesviruses. Glycoprotein gH is dependent on gL for authentic processing and transport, and the complex as a whole has been implicated as important to the ability of virus to fuse with the cell membrane and penetrate into the cytoplasm.

[0084] In some embodiments, the polypeptide construct includes antigenic determinants derived from one or more of the following EBV lytic genes: BLLF1 (gp350/220), BXLF2 (gp85, gH), BKRF2 (gp25, gL), or a variant of any thereof. As discussed *supra*, these EBV genes can also

be categorized as late genes. In some embodiments, the polypeptide construct includes antigenic determinants derived from BLLF1 (gp350/220) or a variant thereof. In some embodiments, the polypeptide construct includes antigenic determinants derived from BXL2F2 (gp85, gH) or a variant thereof. In some embodiments, the polypeptide construct includes antigenic determinants derived from BKRF2 (gp25, gL) or a variant thereof.

[0085] In some embodiments, the polypeptide construct includes antigenic determinants derived from a single EBV antigen (*e.g.*, an EBV polypeptide) or a variant thereof. For example, in some embodiments, the antigenic determinants in the polypeptide construct can be derived from a single EBV lytic gene, a single EBV pre-latent gene, a single EBV latent gene, a single EBV early gene, a single EBV immediate-early gene, a single EBV late gene, or a variant of any thereof. In some embodiments, the antigenic determinants in the polypeptide construct are derived from BLLF1 (gp350/220) or a variant thereof. In some embodiments, the antigenic determinants in the polypeptide construct are derived from BZLF1 or a variant thereof. In some embodiments, the antigenic determinants in the polypeptide construct are derived from LMP2B or a variant thereof.

[0086] In some embodiments, the antigenic determinants in the polypeptide construct are derived from at least two, at least three, at least four, at least five, at least six, or at least seven EBV antigens or variants thereof. In some embodiments, the antigenic determinants in the polypeptide construct are derived from two, three, four, five, six, or seven EBV antigens or variants thereof. In some embodiments, the antigenic determinants in the polypeptide construct are derived from less than two (*e.g.*, one), less than three, less than four, less than five, less than six, or less than seven EBV antigens or variants thereof.

[0087] In some embodiments, the antigenic determinants in the polypeptide construct are derived from an EBV early gene, an EBV latent gene, and an EBV late gene. In some embodiments, the antigenic determinants in the polypeptide construct are derived from an EBV lytic gene, an EBV latent gene, and an EBV latent to lytic reactivation gene. In some embodiments, the antigenic determinants in the polypeptide construct are derived from one or more EBV genes selected from the group consisting of BLLF1 (gp350/220), BZLF1, BXL2F2 (gp85, gH), BKRF2 (gp25, gL), LMP2B, and a combination of any thereof. In some embodiments, the antigenic determinants in the polypeptide construct are derived from one or more EBV genes selected from the group consisting of the following gene combination: BLLF1 (gp350/220),

BZLF1, BXLF2 (gp85, gH), BKRF2 (gp25, gL), and LMP2B (see also, Table 1). Without being bound to any particular theory, antigenic determinants derived from this gene combination are believed to enhance the biological activity of the polypeptide constructs expressed from the srRNA molecules. In particular, it is contemplated that antibodies against gp350 may neutralize B cell entry by EBV, antibodies against gH may neutralize B and/or epithelial cell entry by EBV, and antibodies against gL may generally neutralize B and/or epithelial cell entry (involved in EBV membrane/cell membrane fusion). It is also contemplated that antibodies against LMP2B and BZLF1 may induce anti-EBV T-cells that can attack cells with latent or reactivation of lytic EBV infection, respectively.

TABLE 1: List of EBV antigens included in some exemplary srRNA constructs of the disclosure.

EBV antigen	Viral stage	Modifications	MOA
gp350	Lytic	+/- transmembrane domain in vaccine	Neutralization of B cell entry
gH	Lytic	+/- transmembrane domain in vaccine	Neutralization of B cell and/or epithelial cell entry
gL	Lytic	none	Neutralization of B cell and/or epithelial cell entry
LMP2B	Latent	none	Latency
BZLF1	Latent to lytic reactivation	Excluded regions associated with DNA binding domain and transactivation	Lytic reactivation

[0088] In some embodiments of the disclosure, the coding sequences for the polypeptide construct comprises coding sequences for the following genes: (a) BZLF1 or a variant thereof; (b) BLLF1 (gp350/220) or a variant thereof; (c) LMP2B or a variant thereof; (d) gH and gL, or a variant of any thereof; (e) BZLF1, LMP2B, gH, gL, and BLLF1 (gp350/220), or a variant of any thereof; (f) gH, gL, and BLLF1 (gp350/220), or a variant of any thereof; or (g) BZLF1 and LMP2B, or a variant of any thereof. In some embodiments, the BLLF1 (gp350/220) or variant thereof is devoid of a functional transmembrane (TM) domain.

[0089] In some embodiments, the polypeptide construct includes antigenic determinants derived from a BLLF1 (gp350/220) or variant thereof that is devoid of a functional transmembrane (TM) domain, *e.g.*, a TM domain comprising one or more mutations and/or deletions. Without

being bound to any particular theory or mechanism of action, it is contemplated that a BLLF1 (gp350/220) lacking TM domain poses less of a safety risk than a full-length BLLF1 (gp350/220). Additionally, it is believed that transmembrane domains are generally not the target of neutralizing antibodies. Therefore, the exclusion of transmembrane domains from the polypeptide constructs of the disclosure would, in some cases, help mitigate manufacturing-related size constraints. For example, in some embodiments, the polypeptide construct includes antigenic determinants derived from a BLLF1 (gp350/220) or variant thereof wherein at least a portion of the transmembrane (TM) domain has been deleted. In some embodiments, the TM domain of the BLLF1 (gp350/220) or variant thereof is completely absent.

[0090] In some embodiments, the gH or variant thereof is devoid of a functional transmembrane domain (TM), *e.g.*, a TM domain comprising one or more mutations and/or deletions. Without being bound to any particular theory, it is contemplated that a gH lacking TM domain poses less of a safety risk than a full-length gH. Additionally, it is believed that transmembrane domains are generally not the target of neutralizing antibodies. Therefore, the exclusion of transmembrane domains from the polypeptide constructs of the disclosure would, in some cases, help mitigate manufacturing-related size constraints. For example, in some embodiments, the polypeptide construct includes antigenic determinants derived from a gH or variant thereof wherein at least a portion of the transmembrane (TM) domain has been deleted. In some embodiments, the TM domain of the gH or variant thereof is completely absent.

[0091] In some embodiments, the BZLF1 or variant thereof is devoid of (i) a functional nuclear localization sequence (NLS), and/or (ii) a functional transactivation (TA) domain. Without being bound to any particular theory, it is contemplated that a BZLF1 lacking NLS and TA domain poses less of a safety risk than a full-length BZLF1. For example, in some embodiments, the polypeptide construct includes antigenic determinants derived from BZLF1 or variant thereof comprising one or more mutations and/or deletions in the NLS. In some embodiments, the polypeptide construct includes antigenic determinants derived from a BZLF1 or variant thereof wherein at least a portion of the NLS has been deleted. In some embodiments, the NLS of the BZLF1 or variant thereof is completely absent.

[0092] In some embodiments, the polypeptide construct includes antigenic determinants derived from BZLF1 or variant thereof comprising one or more mutations and/or deletions in the

transactivation (TA) domain. In some embodiments, the polypeptide construct includes antigenic determinants derived from a BZLF1 or variant thereof wherein at least a portion of the TA domain has been deleted. In some embodiments, the TA domain of the BZLF1 or variant thereof is completely absent.

[0093] In some embodiments, the coding sequences for a polypeptide construct include the EBV antigens and the antigen configurations and/or ordinality described in **FIG. 2**. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-34. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 5. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 6. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 7. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 8. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 9. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 10.

[0094] In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 11. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 13. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 14. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 15. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 16. In some

embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 19. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 20.

[0095] In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 21. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 22. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 23. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 24. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 25. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 26. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 28. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 29. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 30.

[0096] In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 31. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 32. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 33. In some embodiments, the polypeptide construct comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 34.

[0097] In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to an amino acid sequence selected from the group consisting of

SEQ ID NOS: 1-34, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 1, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 2, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 3, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 4, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 5, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 6, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 7, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 8, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 9, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 10, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid.

[0098] In some embodiments, the polypeptide construct comprises an amino acid sequence

having 100% sequence identity to SEQ ID NO: 11, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 12, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 13, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 14, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 15, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 16, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 17, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 18, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 19, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 20, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid.

[0099] In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 21, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some

embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 22, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 23, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 24, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 25, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 26, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 27, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 28, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 29, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 30, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid.

[0100] In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 31, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 32, wherein one, two, three, four, five, or more nucleotides of the amino

acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 33, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid. In some embodiments, the polypeptide construct comprises an amino acid sequence having 100% sequence identity to SEQ ID NO: 34, wherein one, two, three, four, five, or more nucleotides of the amino acid sequence may be substituted by a different amino acid.

[0101] The nucleic acid constructs of the disclosure may or may not include coding sequences for antigenic determinants derived from EBNA1 gene. In some particular embodiments, the nucleic acid constructs of the disclosure do not include coding sequences for antigenic determinants derived from a full-length EBNA1 gene. In some other embodiments, the nucleic acid constructs of the disclosure include coding sequences for at least one antigenic determinants derived from a full-length EBNA1 gene.

[0102] The nucleic acid constructs of the disclosure may or may not include coding sequences for antigenic determinants derived from LMP1 gene and/or LMP2A gene. In some particular embodiments, the nucleic acid constructs of the disclosure do not include coding sequences for antigenic determinants derived from a full-length LMP1 gene. In some other embodiments, the nucleic acid constructs of the disclosure include coding sequences for at least one antigenic determinants derived from a full-length LMP1 gene. In some particular embodiments, the nucleic acid constructs of the disclosure do not include coding sequences for antigenic determinants derived from a full-length LMP2A gene. In some other embodiments, the nucleic acid constructs of the disclosure include coding sequences for at least one antigenic determinants derived from a full-length LMP2A gene.

[0103] As described above, in some embodiments of the disclosure, the nucleic acid constructs include a nucleic acid sequence encoding a modified genome or srRNA of an alphavirus, wherein at least a portion of the nucleic acid sequence encoding the viral structural proteins of the modified alphavirus genome or srRNA has been replaced by a coding sequence for a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV). In some embodiments, the modified alphaviral genome or srRNA is devoid of at least a portion of the nucleic acid sequence encoding one or more of the viral structural proteins

CP, E1, E2, E3, and 6K of the unmodified alphaviral genome or srRNA. In some embodiments, the modified viral genome or srRNA is devoid of a portion of or the entire sequence encoding CP. In some embodiments, the modified alphaviral genome or srRNA is devoid of a portion of or the entire sequence encoding E1. In some embodiments, the modified alphaviral genome or srRNA is devoid of a portion of or the entire sequence encoding E2. In some embodiments, the modified alphaviral genome or srRNA is devoid of a portion of or the entire sequence encoding E3. In some embodiments, the modified alphaviral genome or srRNA is devoid of a portion of or the entire sequence encoding 6K. In some embodiments, the modified viral genome or srRNA is devoid of a portion of or the entire sequence encoding a combination of CP, E1, E2, E3, and 6K. Accordingly, some embodiments of the disclosure provide a modified alphaviral genome or srRNA in which the coding sequence for non-structural proteins nsP1, nsP2, nsP3, and nsP4 of the unmodified alphaviral genome or srRNA is present, however at least a portion of or the entire sequence encoding one or more structural proteins (*e.g.*, CP, E1, E2, E3, and 6K) of the alphaviral genome or srRNA is absent.

[0104] In some embodiments, the modified viral genome or srRNA is devoid of a substantial portion of the nucleic acid sequence encoding one or more viral structural proteins. The skilled artisan will understand that a substantial portion of a nucleic acid sequence encoding a viral structural polypeptide can include enough of the nucleic acid sequence encoding the viral structural polypeptide to afford putative identification of that polypeptide, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as The Basic Local Alignment Search Tool (BLAST; publicly available at, for example, guides.lib.berkeley.edu/ncbi/blast and [//blast.ncbi.nlm.nih.gov/Blast.cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi)). Accordingly, a substantial portion of a nucleotide sequence comprises enough of the sequence to afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. For example, a substantial portion of a nucleic acid sequence can include at least about 20%, for example, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95% of the full-length nucleic acid sequence. As described above, the present disclosure provides nucleic acid molecules and constructs which are devoid of partial or complete nucleic acid sequences encoding one or more viral structural proteins. The skilled artisan, having the benefit of the sequences as disclosed herein, can readily use all or a

substantial portion of the disclosed sequences for the compositions and methods of the disclosure. Accordingly, the present application comprises the complete sequences as disclosed herein, *e.g.*, those set forth in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

[0105] In some embodiments, the modified viral genome or srRNA is devoid of the entire sequence encoding viral structural proteins, *e.g.*, the modified viral genome or srRNA includes no nucleic acid sequence encoding the structural proteins of the viral unmodified genome or srRNA.

[0106] In some embodiments, the nucleic acid constructs of the disclosure further include one or more expression cassettes, *e.g.*, for expression of a polypeptide construct of interest, *e.g.*, a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV). In principle, the nucleic acid constructs disclosed herein can generally include any number of expression cassettes. In some embodiments, the nucleic acid constructs disclosed herein can include at least two, at least three, at least four, at least five, or at least six expression cassettes. The skilled artisan will understand that the term “expression cassette” refers to a construct of genetic material that contains coding sequences and enough regulatory information to direct proper transcription and/or translation of the coding sequences in a cell, *in vivo* and/or *ex vivo*. The expression cassette may be inserted into a vector for targeting to a desired host cell and/or into a subject. Accordingly, in some embodiments, the term expression cassette may be used interchangeably with the term “expression construct.” In some embodiments, the term “expression cassette” refers to a nucleic acid construct that includes a gene encoding a protein or functional RNA operably linked to regulatory elements such as, for example, a promoter and/or a termination signal, and optionally, any or a combination of other nucleic acid sequences that affect the transcription or translation of the gene.

[0107] In some embodiments, at least one of the expression cassettes includes a promoter operably linked to a heterologous nucleic acid sequence. In some embodiments, the heterologous nucleic acid sequence may encode a polypeptide construct of interest, *e.g.*, a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV). Accordingly, the nucleic acid constructs as provided herein can find use, for example, as an expression vector that, when including a regulatory element (*e.g.*, a promoter) operably linked to a heterologous nucleic acid sequence, can affect expression of the heterologous nucleic acid

sequence (*e.g.*, heterologous nucleic acid sequence encoding a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV). Both naturally-occurring promoter sequences and synthetically-designed (and/or produced) promoter sequences are suitable. Accordingly, in some embodiments, the promoter is or comprises naturally occurring sequences isolated from or derived from a genomic sequence of a gene. In some embodiments, the promoter may be synthetically produced or designed by altering known DNA elements. In some embodiments, at least one of the expression cassettes includes a subgenomic (*sg*) promoter operably linked to a heterologous nucleic acid sequence. In some embodiments, the *sg* promoter is a 26S subgenomic promoter. In some embodiments, at least one nonstructural protein (nsP), or a portion thereof, of the modified alphaviral genome or srRNA is heterologous relative to the remainder of the modified alphaviral genome or srRNA. In some embodiments, the modified alphaviral genome or srRNA further includes a nucleic acid sequence encoding a heterologous nsP or a portion thereof. In some embodiments, the nucleic acid molecules of the disclosure further include one or more untranslated regions (UTRs). In some embodiments, at least one of the UTRs is a heterologous UTR. For example, in some embodiments, the nucleic acid molecules of the disclosure can include a native 5' UTR and a heterologous 3' UTR. In some embodiments, the nucleic acid molecules of the disclosure can include a heterologous 5' UTR and a native 3' UTR. . In some embodiments, both 5' UTR and 3' UTR are heterologous relative to the remainder of the modified alphaviral genome or srRNA.

[0108] In some embodiments, at least one of expression cassettes includes a coding sequence for a gene of interest (GOI). In some embodiments, the coding sequence for the GOI includes a coding sequence for a polypeptide construct of interest (PCI). In some embodiments, the polypeptide construct of interest includes a single EBV polypeptide sequence (*e.g.*, monogenic PCI). In some embodiments, the coding sequence for the PCI includes coding sequences for a plurality of EBV polypeptides, *e.g.*, multigenic PCI (*e.g.*, bigenic, trigenic, or tetragenic, *etc.*). In some embodiments, each of the coding sequences of the plurality of polypeptides is operably linked to a separate promoter sequence. In some embodiments, the coding sequences of the plurality of polypeptides are operably linked to one another within a single open reading frame, *e.g.*, in a polycistronic ORF (see, *e.g.*, Example 1, Rep-674, Rep-675, Rep-678, Rep-679, Rep-680, Rep-695, Rep-728, Rep-729, Rep-730, Rep-731, and Rep-733). In some embodiments, the coding

sequences of the plurality of polypeptides are configured into two or more ORFs that are connected to one another by a sequence for an internal ribosomal entry site (IRES) (see, *e.g.*, Example 1, Rep-692, Rep-693, Rep-694, Rep-696, Rep-697, Rep-698, Rep-699, Rep-734, Rep-735, Rep-736, and Rep-737).

[0109] In some embodiments, the coding sequence of the polycistronic ORF(s) is operably linked to a promoter sequence. In some embodiments, at least one of the promoter sequences is a subgenomic (*sg*) promoter. In some embodiments, the *sg* promoter is a 26S genomic promoter.

[0110] In some embodiments, the plurality of EBV polypeptides can be linked to one another directly or indirectly (*e.g.*, via one or more connector sequences, for example via linkers). For example, in some embodiments, the plurality of EBV polypeptides can be directly linked to one another, *e.g.*, adjacently to one another. In some embodiments, at least two (*e.g.*, 2, 3, 4, or 5) of the plurality of EBV polypeptides are operably linked to one another by one or more connector sequences (*e.g.*, linkers). In some embodiments, the length and amino acid composition of the connector sequences can be optimized to vary the orientation, flexibility, and/or proximity of the polypeptides relative to one another to achieve a desired activity or property of the PCI. In some embodiments, a connector sequence of the plurality of connector sequences (*e.g.*, linkers) includes one or more autoproteolytic peptide sequences. Non-limiting examples of autoproteolytic peptide sequences suitable for the methods and compositions of the disclosure include autoproteolytic cleavage sequences derived from calcium-dependent serine endoprotease (furin), porcine teschovirus-1 2A (P2A), foot-and-mouth disease virus (FMDV) 2A (F2A), Equine Rhinitis A Virus (ERAV) 2A (E2A), Thosea asigna virus 2A (T2A), cytoplasmic polyhedrosis virus 2A (BmCPV2A), and Flacherie Virus 2A (BmIFV2A). In some embodiments, the polypeptide constructs of the disclosure include one or more P2A autoproteolytic cleavage sequences. In some embodiments, at least two of the plurality of polypeptides are operably linked to each other via a P2A autoproteolytic cleavage sequence.

[0111] In some embodiments, the coding sequences of the plurality of polypeptides and their ordinality are configured as shown in FIG. 2. In some embodiments, the coding sequence for the PCI includes coding sequences for (i) gH, (ii) gL, or a variant of any thereof. In some embodiments, the coding sequences for (i) gH, (ii) gL, or a variant of any thereof are operably linked to one another within a single polycistronic ORF.

[0112] In some embodiments, the coding sequence for the PCI includes coding sequences for (i) gH, (ii) gL, (iii) gp350, or a variant of any thereof. In some embodiments, the coding sequences for (i) gH, (ii) gL, (iii) gp350, or a variant of any thereof are operably linked to one another within a single polycistronic ORF.

[0113] In some embodiments, the coding sequence for the PCI includes coding sequences for (i) BZLF1, (ii) gH, (iii) gL, (iv) gp350, and (v) LMP2B, or a variant of any thereof. In some embodiments, the coding sequences for (i) BZLF1, (ii) gH, (iii) gL, (iv) gp350, and (v) LMP2B, or a variant of any thereof are operably linked to one another within a single polycistronic ORF.

[0114] In some embodiments, the coding sequences for (i) BZLF1, (ii) gH, (iii) gL, (iv) gp350, and (v) LMP2B, or a variant of any thereof are configured into a first ORF and a second ORF that are connected to one another by an IRES. The designation of the ORFs within the coding sequence for a PCI describe herein as the “first,” “second,” “third,” or “fourth” ORFs is not intended to imply any particular structural arrangement of the “first,” “second,” “third,” or “fourth” ORFs within the PCI. In some embodiments, the first ORF includes coding sequences for BZLF1 or a variant thereof; and the second ORF includes coding sequences for (i) gH, (ii) gL, (iii) gp350, and (iv) LMP2B or a variant of any thereof. In some embodiments, the first ORF includes coding sequences for (i) BZLF1 and (ii) gH, and (iii) gL, or a variant of any thereof; and the second ORF includes coding sequences for (i) gp350, and (ii) LMP2B, or a variant of any thereof.

[0115] In some embodiments, the first ORF includes coding sequences for (i) BZLF1, (ii) gH, and (iii) gL, or a variant any thereof; and the second ORF includes coding sequences for (i) gp350 and (ii) LMP2B or a variant of any thereof.

[0116] In some embodiments, the first ORF includes coding sequences for (i) BZLF1, (ii) gH, (iii) gL, and (iv) gp350, or a variant of any thereof; and the second ORF includes coding sequences for LMP2B or a variant thereof.

[0117] In some embodiments, the first ORF includes coding sequences for (i) gH, (ii) gL, and (iii) gp350, or a variant of any thereof; and the second ORF includes coding sequences for (i) BZLF1 and (ii) LMP2B or a variant of any thereof. In some embodiments, the first ORF includes coding sequences for (i) gH, (ii) gL, and (iii) gp350, or a variant or any thereof; and the second ORF includes coding sequences for (i) LMP2B and (ii) BZLF1 or a variant of any thereof. In some embodiments, the first ORF includes coding sequences for (i) gH, (ii) gL, (iii) gp350, and (iv)

LMP2B, or a variant or any thereof; and the second ORF includes coding sequences for BZLF1 or a variant thereof.

[0118] In some embodiments, the first ORF includes coding sequences for (i) gp350, (ii) gH, (iii) gL, and (iv) LMP2B, or a variant or any thereof; and the second ORF includes coding sequences BZLF1 or a variant thereof.

[0119] In some embodiments, the first ORF includes coding sequences for gp350 or a variant thereof; and the second ORF includes coding sequences for (i) gH and (ii) gL, or a variant of any thereof. In some embodiments, the first ORF includes coding sequences for (i) gL and (ii) gH, or a variant of any thereof; and the second ORF includes coding sequences for gp350 or a variant thereof. In some embodiments, the first ORF includes coding sequences for gp350 or a variant thereof; and the second ORF includes coding sequences for (i) gL and (ii) gH, or a variant of any thereof.

[0120] In some embodiments, the first ORF includes coding sequences for LMP2B or a variant thereof; and the second ORF includes coding sequences for BZLF1 or a variant thereof. In some embodiments, the first ORF includes coding sequences for BZLF1 or a variant thereof; and the second ORF includes coding sequences for LMP2B or a variant thereof.

[0121] In some embodiments, the gp350- and/or gH- sequences lack a functional transmembrane domain (-TM). In some embodiments, the sequences of BZLF1 is devoid of (i) a functional nuclear localization sequence (NLS) and (ii) a functional transactivation (TA) domain.

[0122] There are no particular limitations in regard to EBV strains suitable for the compositions and methods of the disclosure. The strain of EBV used in the compositions and methods disclosed herein may be any strain of EBV. Virulent EBV strains and avirulent EBV strains are both suitable. In some embodiments, the one or more of EBV antigenic determinants is of an EBV type 1 (EBV-1), or an EBV type 2 (EBV-2), or a combination thereof. Non-limiting examples of EBV strains suitable for the compositions and methods of the disclosure include AG876 strain, Akata strain, Alaska strain, B95-8 strain, Hina 1 strain, HKNPC strain, China 2 strain, CVI 988 strain, GD1 strain, GD2 strain, GP202 strain, M81 strain, Mediterranean strain, Mutu strain, P3HR1 strain, Raji strain, SNU-719 strain, YCCEL1 strain, or a combination thereof.

[0123] In some embodiments, the coding sequences of the plurality of polypeptides are operably linked to one another by one or more internal ribosomal entry sites (IRES). Non-limiting

examples of IRES suitable for the methods and compositions of the disclosure include viral IRES sequences, cellular IRES sequences, and artificial IRES sequences. Examples of suitable IRES sequences include, but are not limited to, Kaposi's sarcoma-associated herpesvirus (KSHV) IRES, hepatitis virus IRES, Pestivirus IRES, Cripavirus IRES, Rhopalosiphum padi virus IRES, fibroblast growth factor IRES, platelet-derived growth factor IRES, vascular endothelial growth factor IRES, insulin-like growth factor IRES, picornavirus IRES, encephalomyocarditis virus (EMCV) IRES, Pim-1 IRES, p53 IRES, Apaf-1 IRES, TDP2 IRES, L-myc IRES, and c-myc IRES. In some embodiments, the internal ribosomal entry site (IRES) is from encephalomyocarditis virus (EMCV). In some embodiments, the one or more IRES sequences comprises the sequence of SEQ ID NO: 58.

[0124] In some embodiments of the methods described herein, the recombinant alphavirus srRNA is of a virus belonging to the *Alphavirus* genus of the *Togaviridae* family. In some embodiments of the disclosure, the modified alphavirus genome or srRNA is of an alphavirus belonging to the Venezuelan equine encephalitis virus/Eastern Equine Encephalitis virus (VEEV/EEEV) group, or the Semliki Forest virus (SFV) group, or the Sindbis virus (SINV) group. In some embodiments, the modified alphavirus genome or srRNA is of an alphavirus belonging to the BFV complex, EEEV complex, MIDV complex, NDUV complex, SFV complex, VEEV complex, WEEV complex. In some embodiments, 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), Sagiya 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. In some embodiments, the alphavirus is VEEV, EEEV, CHIKV, or SINV. In some embodiments, the alphavirus is VEEV. In some embodiments, the alphavirus is EEEV. In some embodiments, the alphavirus is Western Equine Encephalitis virus (WEEV). In some embodiments, the alphavirus is CHIKV. In some embodiments, the alphavirus is SINV.

[0125] In some embodiments, the alphavirus is Chikungunya virus (CHIKV). Non-limiting

examples of CHIKV strains suitable for the compositions and methods of the disclosure include CHIKV S27, CHIKV LR2006-OPY-1, CHIKV YO123223, CHIKV DRDE, CHIKV 37997, CHIKV 99653, CHIKV Ag41855, and Nagpur (India) 653496 strain. Virulent and avirulent CHIKV strains are both suitable. Additional examples of CHIKV strains suitable for the compositions and methods of the disclosure include but are not limited to those described in Afreen *et al. Microbiol. Immunol.* 2014, 58:688-696, Lanciotti and Lambert *ASTMH* 2016, 94(4):800-803 and Langsjoen *et al. mBio.* 2018, 9(2):e02449-17. In some embodiments, the modified CHIKV genome or replicon RNA (*e.g.*, self-replicating RNA) is derived from CHIKV strain S27. In some embodiments, the modified CHIKV genome or replicon RNA is derived from CHIKV strain DRDE. In some embodiments, the modified CHIKV genome or replicon RNA is derived from CHIKV strain DRDE-06. In some embodiments, the modified CHIKV genome or replicon RNA is derived from CHIKV strain DRDE-07. In some embodiments, the modified CHIKV genome or replicon RNA is derived from CHIKV strain S27.

[0126] In some embodiments, the alphavirus is Eastern Equine Encephalitis virus (EEEV). Non-limiting examples of EEEV strains suitable for the compositions and methods of the disclosure include EEEV 792138, 783372, BeAn5122, BeAr300851, BeAr436087, C-49, FL91-4679, FL93-939, GML903836, MP-9, PE6, and V105-00210. Virulent and avirulent EEEV strains are both suitable. Additional suitable EEEV strains include, but are not limited to those described in the Virus Pathogen Resource website (ViPR; which is publicly available at www.viprbrc.org/brc/vipr_genome_search.spg?method=SubmitForm&blockId=868&decorator=to ga). In some embodiments, the modified EEEV genome or replicon RNA (*e.g.*, self-replicating RNA) is derived from EEEV strain FL93-939.

[0127] In some embodiments, the alphavirus is Sindbis virus (SINV). In some embodiments, the modified genome or RNA replicon (*e.g.*, self-replicating RNA) is of a SINV strain. Non-limiting examples of SINV strains suitable for the compositions and methods of the disclosure include SINV strain AR339, AR86, and Girdwood. Examples of SINV strains suitable for the compositions and methods of the disclosure include, but are not limited to those described in Sammels *et al. J. Gen. Virol.* 1999, 80(3):739-748, Lundström and Pfeffer *Vector Borne Zoonotic Dis.* 2010, 10(9):889-907, Sigei *et al. Arch. of Virol.* 2018, 163:2465-2469 and Ling *et al. J. Virol.* 2019, 93:e00620-19. Additional suitable SINV strains include, but are not limited to those

described in the Virus Pathogen Resource website (ViPR; which is publicly available at www.viprbrc.org/brc/vipr_genome_search.spg?method=SubmitForm&blockId=868&decorator=toga). Virulent and avirulent SINV strains are both suitable. In some embodiments, the modified genome or RNA replicon is of a SINV strain Girdwood. In some embodiments, the modified genome or RNA replicon is of a SINV strain AR86. In some embodiments, the modified SINV genome or replicon RNA is derived from SINV strain Girdwood. In some embodiments, the modified SINV genome or replicon RNA is derived from SINV strain AR86. In some embodiments, the at least one heterologous nsP or portion thereof of the modified genome or RNA replicon is derived from a SINV strain AR86. In some embodiments, the at least one heterologous nsP or portion thereof is nsP1, nsP3, nsP4, or a portion of any thereof, or a combination of any of the foregoing. In some embodiments, the modified genome or RNA replicon is of a SINV strain AR86.

[0128] In some embodiments, the alphavirus is Western Equine Encephalitis virus (WEEV). Non-limiting examples of WEEV strains suitable for the compositions and methods of the disclosure include WEEV California, McMillan, IMP181, Imperial, Imperial 181, IMPR441, 71V-1658, AG80-646, BFS932, COA592, EP-6, E1416, BFS1703, BFS2005, BSF3060, BSF09997, CHLV53, KERN5547, 85452NM, Montana-64, S8-122, and TBT-235. Additional examples of WEEV strains suitable for the compositions and methods of the disclosure include 5614, 93A27, 93A30, 93A38, 93A79, B628(CI 15), CBA87, CNTR34, CO921356, Fleming, Lake43, PV012357A, PV02808A, PV72102, R02PV001807A, R02PV002957B, R02PV003422B, R05PV003422B, R0PV003814A and R0PV00384A. Virulent and avirulent WEEV strains are both suitable. Additional suitable WEEV strains include, but are not limited to those described in Bergren NA *et al.*, J. Virol. 88(16): 9260–9267, Aug 2014, and in the Virus Pathogen Resource website (ViPR; which is publicly available at https://www.viprbrc.org/brc/vipr_genome_search.spg?method=SubmitForm&blockId=57240&decorator=toga). In some embodiments, the modified WEEV genome or srRNA is derived from WEEV strain Imperial. In some embodiments, the modified WEEV genome or srRNA is derived from WEEV strain McMillan.

[0129] In some embodiments, the alphavirus is Madariaga virus (MADV), formerly referred to as South American Eastern Equine Encephalitis virus (SA EEEV). Non-limiting examples of

MADV strains suitable for the compositions and methods of the disclosure include ArgLL, ArgB, BeAn-5122, ArgM, 24443 (TR59), 25714 (BG60), BeAr 18205, 900188 (PA62), BeAr 81828, BeAr 126650, 68U231, 77U1104 (PE70), 75V1496, BeAr 300851, 75U40, and El Delirio (Arrigo NC *et al.*, supra 2010). Additional examples of MADV strains suitable for the compositions and methods of the disclosure include 76V25343, 77U1 (BR77), BeAr348998, IVICPan57151, BeAn416361, 903836 (PA84), BeAr436087, 435731 (PA86), C49 (CO92), PE-0.0155-96 (0.0155), PE-3.0815-96 (3.0815), PE-16.0050-98 (16.0050), PE-18.0140-99 (18.0140), and PE-18.0172-99 (18.0172) (Arrigo NC *et al.*, supra 2010). Additional suitable MADV strains include, but are not limited to those described in Arrigo NC *et al.*, supra 2010, and in the Virus Pathogen Resource website (ViPR; which is publicly available at www.viprbrc.org/brc/vipr_genome_search.spg?method=SubmitForm&blockId=868&decorator=to_ga). In some embodiments, the modified MADV genome or srRNA is derived from MADV strain BeAr300851.

[0130] In some embodiments, the srRNA constructs of the disclosure can include a 5' cap. The term "5' cap" as used herein refers to a structure found on the 5' end of some eukaryotic RNAs, e.g., RNA transcripts, and generally includes a dinucleotide or a guanosine nucleotide connected to an RNA, e.g., mRNA, via a 5'- to 5'-triphosphate linkage (also referred to as Gppp or G(5')ppp(5')). In some embodiments, a guanosine nucleoside included in a 5' cap may be modified, for example, by methylation at one or more positions (e.g., at the 7-position) on a base (guanine), and/or by methylation at one or more positions of a ribose. In some embodiments, a guanosine nucleoside included in a 5' cap includes a 2'O methylation at a ribose (2'OMeG). In some embodiments, a guanosine nucleoside included in a 5' cap includes methylation at the 7-position of guanine (m7G). In some embodiments, a guanosine nucleoside included in a 5' cap includes methylation at the 7-position of guanine and a 2' O methylation at a ribose (m7(2'OMeG)).

[0131] Multiple distinct cap structures can be used to generate the 5' cap of *in vitro* transcribed synthetic srRNA. In some embodiments, providing an srRNA with a 5' cap disclosed herein or a 5' cap analog may be achieved by *in vitro* transcription, in which a 5' cap is co-transcriptionally expressed into an RNA strand, or may be attached to an RNA post-transcriptionally using capping enzymes. Accordingly, in some embodiments, 5' capping of synthetic srRNA can be performed co-transcriptionally with chemical cap analogs (i.e., capping

during *in vitro* transcription). For example, CleanCap® technology provides high efficiency capping (90%+) in a co-transcriptional reaction using commercially available reagents with an AG initiator to provide a natural Cap 1 structure with a 2'-O-methyl group and N7 methyl on separate guanine components. As another example, the Anti-Reverse Cap Analog (ARCA) cap contains a 5'-5'-triphosphate guanine-guanine linkage where one guanine contains an N7 methyl group as well as a 2'-O-methyl group. Alternatively, in some embodiments, synthetic srRNA molecules may also be enzymatically capped post-transcriptionally. These may generate a more authentic 5' cap structure that more closely mimics, either structurally or functionally, the endogenous 5' cap which have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5' endonucleases and/or reduced 5' decapping. Numerous synthetic 5' cap analogs have been developed and are known in the art to enhance mRNA stability and translatability (see, e.g., Grudzien-Nogalska E. et al., Synthetic mRNAs with superior translation and stability properties in Synthetic Messenger RNA and Cell Metabolism Modulation in Methods in Molecular Biology v.969 (Rabinovich, P. H. Ed), 2013).

[0132] Thus, in some embodiments, the srRNA of the disclosure is co-transcriptionally capped srRNA. Exemplary co-transcriptionally capped srRNAs include, without limitation, anti-reverse cap analogs (ARCAs) and CleanCap® srRNA.

[0133] In other embodiments, the srRNA of the disclosure is enzymatically capped srRNA. Exemplary capping enzymes include, without limitation, Vaccinia virus capping enzyme (VCE) and Faustovirus capping enzyme (FCE).

[0134] In some embodiments, the modified alphavirus genome or srRNA is of an alphavirus belonging to the VEEV/EEEV group, or the SFV group, or the SINV group. In some embodiments, the alphavirus is Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV), Chikungunya virus (CHIKV), Madariaga virus (MADV), Western equine encephalitis virus (WEEV), or Sindbis virus (SINV).

[0135] In some embodiments, the coding sequence of the polypeptide construct of interest (PCI) can be redesigned and/or optimized for a desired property, such as increased stability, potency, and expression (e.g., translation efficiency), which in turns can maximize the impact of producing, delivering, and administering biotherapeutic. For example, in some embodiments, the coding sequence of the PCI is optimized for expression at a level higher than the expression level

of a reference coding sequence. With respect to sequence-optimization of nucleotide sequences, degeneracy of the genetic code provides the possibility to substitute at least one base of the protein encoding sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. Hence, the nucleic acid constructs of the present disclosure may also have any base sequence that has been changed from any polynucleotide sequence disclosed herein by substitution in accordance with degeneracy of the genetic code. References describing codon usage are readily publicly available. In some embodiments, polynucleotide sequence variants can be produced for a variety of reasons, *e.g.*, to optimize expression for a particular host (*e.g.*, changing codon usage in the alphavirus mRNA to those preferred by other organisms such as human, non-human primates, hamster, mice, or monkey). Accordingly, in some embodiments, the coding sequence of the PCI is optimized for expression in a target host cell through the use of codons optimized for expression. The techniques for the construction of synthetic nucleic acid sequences encoding PCI using preferred codons optimal for host cell expression may be determined by computational methods analyzing the commonality of codon usage for encoding native proteins of the host cell genome and their relative abundance by techniques well known in the art. The codon usage database (<http://www.kazusa.or.jp/codon>) may be used for generation of codon optimized sequences in mammalian cell environments. Furthermore, a variety of software tools are available to convert sequences from one organism to the optimal codon usage for a different host organism such as the JCat Codon Optimization Tool (www.jcat.de), Integrated DNA Technologies (IDT) Codon Optimization Tool (<https://www.idtdna.com/CodonOpt>) or the Optimizer online codon optimization tool (<http://genomes.urv.es/OPTIMIZER>). Such synthetic sequences may be constructed by techniques known in the art for the construction of synthetic nucleic acid molecules and may be obtained from a variety of commercial vendors. Accordingly, in some embodiments, the coding sequence of the PCI is optimized for expression at a level higher than the expression level of a reference coding sequence, such as, for example, a coding sequence that has not been codon-optimized. In some embodiments, the codon-optimized sequence of the PCI results in an increased expression level by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% compared to a reference coding sequence that has not been codon-optimized. In some embodiments, the codon-optimized sequence of the

PCI results in an increased expression level by at least 2-fold, at least 3-fold, at least 4-fold, or at least 5-fold compared to a reference coding sequence that has not been codon-optimized.

[0136] In some embodiments, the coding sequence of the PCI is optimized for enhanced RNA stability and/or expression. The stability of RNA generally relates to the “half-life” of RNA. “Half-life” relates to the period of time which is needed to eliminate half of the activity, amount, or number of molecules. In the context of the present disclosure, the half-life of an RNA is indicative for the stability of said RNA. The half-life of RNA may influence the “duration of expression” of the RNA. Additional information regarding principles, strategies, and methods for use in enhancing RNA stability can be found in, for example, Leppek K. et al., *Nat. Commun.* 13, 1536 (2022).

[0137] In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence encoding a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV) and having 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 a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 35-56.

[0138] In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 35. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 36. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 37. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 38. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 39. In some embodiments, the nucleic acid sequence encoding the

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: 50. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 51. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 52. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 53. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 54. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 55. In some embodiments, the nucleic acid sequence encoding the polypeptide construct 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: 56.

[0140] In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence encoding a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV) and having 100% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 35-56, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 35, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 36, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some

embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 37, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 38, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 39, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 40, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 41, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 42, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 43, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 44, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 45, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 46, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide.

[0141] In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 47, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 48, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 49, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 50, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 51, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 52, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 53, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 54, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 55, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide. In some embodiments, the nucleic acid constructs of the disclosure include a nucleic acid sequence having 100% sequence identity to SEQ ID NO: 56, wherein one, two, three, four, five, or more nucleotides of the nucleic acid sequence may be substituted by a different nucleotide.

[0142] Nucleic acid sequences having a high degree of sequence identity (*e.g.*, 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%) to a sequence selected from the group consisting of SEQ ID NOS: 35-56 can be identified and/or isolated by using the sequences identified herein (*e.g.*, SEQ ID NOS: 35-56) or any others as they are known in the art, by viral genome sequence analysis, hybridization, and/or PCR with degenerate primers or gene-specific primers from sequences identified in the respective SEQ ID NOS.

[0143] The molecular techniques and methods by which these new nucleic acid constructs were assembled and characterized are described more fully in the Examples of the present application. In some embodiments, the nucleic acid molecules disclosed herein can be produced using recombinant DNA technology (*e.g.*, polymerase chain reaction (PCR) amplification, cloning, *etc.*) or chemical synthesis. Nucleic acid molecules as disclosed herein include natural nucleic acid molecules and homologs thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which one or more nucleotide residues have been inserted, deleted, and/or substituted, in such a manner that such modifications provide the desired property in effecting a biological activity as described herein. Accordingly, in some embodiments, the nucleic acid molecules are recombinant nucleic acid molecules.

[0144] One skilled in the art will appreciate that nucleic acid molecules, including variants of naturally-occurring nucleic acid sequences, can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook *et al.*, In: Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989)). The sequence of a nucleic acid molecule can be modified with respect to a naturally-occurring sequence from which it is derived using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as but not limited to site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, PCR amplification and/or mutagenesis of selected regions of a nucleic acid sequence, recombinational cloning, and chemical synthesis, including chemical synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected from a mixture of modified nucleic acid molecules by screening for the function of

the protein or the srRNA encoded by the nucleic acid molecule and/or by hybridization with a wild-type gene or fragment thereof, or by PCR using primers having homology to a target or wild-type nucleic acid molecule or sequence.

B. Recombinant cells

[0145] The nucleic acid constructs of the present disclosure can be introduced into a host cell or an animal to produce a recombinant cell or transgenic animal containing the nucleic acid molecule. Accordingly, prokaryotic or eukaryotic cells that contain a nucleic acid construct encoding a modified alphavirus genome or srRNA as described herein are also features of the disclosure. In a related aspect, some embodiments disclosed herein relate to introducing into a host cell, such as an animal cell, a nucleic acid construct as provided herein, and then selecting or screening for a transformed cell and/or transgenic animals. Introduction of the nucleic acid constructs of the disclosure into cells and/or animals can be achieved by methods known to those skilled in the art such as, for example, viral infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, nucleofection, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, direct micro-injection, nanoparticle-mediated nucleic acid delivery, and the like.

[0146] Accordingly, some embodiments of the disclosure relate to recombinant cells, for example, recombinant animal cells that include a nucleic acid construct described herein. The nucleic acid construct can be stably integrated in the host genome, or can be episomally replicating, or present in the recombinant host cell as a mini-circle expression vector for a stable or transient expression. Accordingly, in some embodiments of the disclosure, the nucleic acid construct is maintained and replicated in the recombinant host cell as an episomal unit. In some embodiments, the nucleic acid construct is stably integrated into the genome of the recombinant cell. Stable integration can be completed using classical random genomic recombination techniques or with more precise genome editing techniques such as using guide RNA directed CRISPR/Cas9 or TALEN genome editing. In some embodiments, the nucleic acid construct present in the recombinant host cell as a mini-circle expression vector for a stable or transient expression. In some embodiments, the recombinant cells and/or transgenic animals include an srRNA molecule as described herein, *e.g.*, RNA molecule that contains all of the genetic

information required for directing its own amplification or self-replication within a permissive cell and/or animal.

[0147] In some embodiments, the recombinant cell is a prokaryotic cell, such as the bacterium *E. coli*, or a eukaryotic cell, such as an insect cell (*e.g.*, a mosquito cell or a Sf21 cell), or mammalian cells (*e.g.*, COS cells, NIH 3T3 cells, or HeLa cells). In some embodiments, the cell is *in vivo*. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is *in vitro*. In some embodiments, the recombinant cell is a eukaryotic cell. In some embodiments, the recombinant cell is an animal cell. In some embodiments, the animal cell is a vertebrate animal cell or an invertebrate animal cell. In some embodiments, the recombinant cell is a mammalian cell. Non-limiting examples of recombinant cells suitable for the methods and compositions of the disclosure include monkey kidney CV1 cells transformed by SV40 (*e.g.*, COS-7 cells), human embryonic kidney cells (*e.g.*, HEK 293 or HEK 293 cells) or derivative cells thereof (*e.g.*, BHK-21 or BHK-570 cells), baby hamster kidney cells (BHK), mouse sertoli cells (*e.g.*, TM4 cells), monkey kidney cells (*e.g.*, CV1 cells), human cervical carcinoma cells (*e.g.*, HeLa cells), canine kidney cells (MDCK cells), buffalo rat liver cells (*e.g.*, BRL 3A cells), human lung cell (*e.g.*, W138 cells), human liver cell (*e.g.*, Hep G2 cells), mouse mammary tumor (*e.g.*, MMT 060562 cells), TRI cells, FS4 cells, Chinese hamster ovary cells (CHO cells), African green monkey kidney cell (*e.g.*, Vero cells), human A549 cells, human cervix cells, human CHME5 cells, human PER.C6 cells, NS0 murine myeloma cells, human epidermoid larynx cells, human fibroblast cells, human HUH-7 cells, human MRC-5 cells, human muscle cells, human endothelial cells, human astrocyte cells, human macrophage cells, human RAW 264.7 cells, mouse 3T3 cells, mouse L929 cells, mouse connective tissue cells, mouse muscle cells, and rabbit kidney cells. In some embodiments, the recombinant cell is a cell derived from a cell described above (*i.e.*, a derivative cell of an original cell described herein) such as, for example, a cell that is either expanded from a clone of the original cell, an engineered version of the original cell, or a reclassification of the original cell after it has undergone extensive passaging, or has been passaged through another host.

[0148] In some embodiments, the recombinant cell is an insect cell, *e.g.*, cell of an insect cell line. In some embodiments, the recombinant insect cell is a Sf21 cell. Additional suitable insect cell lines include, but are not limited to, cell lines established from insect orders Diptera, Lepidoptera and Hemiptera, and can be derived from different tissue sources. In some

embodiments, the recombinant cell is a cell of a lepidopteran insect cell line. In the past few decades, the availability of lepidopteran insect cell lines has increased at about 50 lines per decade. More information regarding available lepidopteran insect cell lines can be found in, *e.g.*, Lynn D.E., *Available lepidopteran insect cell lines*. Methods Mol Biol. 2007;388:117-38, which is herein incorporated by reference. In some embodiments, the recombinant cell is a mosquito cell, *e.g.*, a cell of mosquito species within *Anopheles* (*An.*), *Culex* (*Cx.*) and *Aedes* (*Stegomyia*) (*Ae.*) genera. Exemplary mosquito cell lines suitable for the compositions and methods described herein include cell lines from the following mosquito species: *Aedes aegypti*, *Aedes albopictus*, *Aedes pseudoscutellaris*, *Aedes triseriatus*, *Aedes vexans*, *Anopheles gambiae*, *Anopheles stephensi*, *Anopheles albimanus*, *Culex quinquefasciatus*, *Culex theileri*, *Culex tritaeniorhynchus*, *Culex bitaeniorhynchus*, and *Toxorhynchites amboinensis*. Suitable mosquito cell lines include, but are not limited to, CCL-125, Aag-2, RML-12, C6/26, C6/36, C7-10, AP-61, A.t. GRIP-1, A.t. GRIP-2, UM-AVE1, Mos.55, Sua1B, 4a-3B, Mos.43, MSQ43, and LSB-AA695BB. In some embodiments, the mosquito cell is a cell of a C6/26 cell line.

[0149] In another aspect, provided herein are cell cultures including at least one recombinant cell as disclosed herein, and a culture medium. Generally, the culture medium can be any suitable culture medium for culturing the cells described herein. Techniques for transforming a wide variety of the above-mentioned host cells and species are known in the art and described in the technical and scientific literature. Accordingly, cell cultures including at least one recombinant cell as disclosed herein are also within the scope of this application. Methods and systems suitable for generating and maintaining cell cultures are known in the art.

C. Transgenic animals

[0150] Also provided, in another aspect, are transgenic animals including a nucleic acid construct as described herein. In some embodiments, the transgenic animal is a vertebrate animal or an invertebrate animal. In some embodiments, the transgenic animal is an insect. In some embodiments, the insect is a mosquito. In some embodiments, the transgenic animal is a mammalian. In some embodiments, the transgenic mammalian is a non-human mammalian. In some embodiments, the transgenic animal produces the polypeptide constructs, EBV antigens, and/or EBV antigenic determinants as describes herein.

[0151] The transgenic non-human host animals of the disclosure are prepared using standard methods known in the art for introducing exogenous nucleic acid into the genome of a non-human animal. In some embodiments, the non-human animals of the disclosure are non-human primates. Other animal species suitable for the compositions and methods of the disclosure include animals that are (i) suitable for transgenesis and (ii) capable of rearranging immunoglobulin gene segments to produce an antibody response. Examples of such species include but are not limited to mice, rats, hamsters, rabbits, chickens, goats, pigs, sheep and cows. Approaches and methods for preparing transgenic non-human animals are known in the art. Exemplary methods include pronuclear microinjection, DNA microinjection, lentiviral vector mediated DNA transfer into early embryos and sperm-mediated transgenesis, adenovirus mediated introduction of DNA into animal sperm (*e.g.*, in pig), retroviral vectors (*e.g.*, avian species), somatic cell nuclear transfer (*e.g.*, in goats). The state of the art in the preparation of transgenic domestic farm animals is reviewed in Niemann, H. *et al.* (2005) *Rev. Sci. Tech.* 24:285-298.

[0152] In some embodiments, the animal is a vertebrate animal or an invertebrate animal. In some embodiments, the animal is an insect. In some embodiments, the insect is a mosquito. In some embodiments, the animal is a mammalian subject. In some embodiments, the mammalian animal is a non-human animal. In some embodiments, the mammalian animal is a non-human primate. In some embodiments, the transgenic animals of the disclosure can be made using classical random genomic recombination techniques or with more precise techniques such as guide RNA-directed CRISPR/Cas genome editing, or DNA-guided endonuclease genome editing with NgAgo (*Natronobacterium gregoryi* Argonaute), or TALENs genome editing (transcription activator-like effector nucleases). In some embodiments, the transgenic animals of the disclosure may be made using transgenic microinjection technology and do not require the use of homologous recombination technology and thus are considered to be easier to prepare and select than approaches using homologous recombination.

D. Pharmaceutical compositions

[0153] The nucleic acid constructs and/or recombinant cells of the disclosure can be incorporated into compositions, including pharmaceutical compositions. Such compositions generally include one or more of the nucleic acid constructs and/or recombinant cells described and provided herein, and a pharmaceutically acceptable excipient, *e.g.*, carrier. In some

embodiments, the compositions of the disclosure are formulated for the prevention, treatment, or management of a health condition such as an EBV-associated disease or infection (e.g., EBV infection). For example, the compositions of the disclosure can be formulated as a prophylactic composition, a therapeutic composition, or a pharmaceutical composition comprising a pharmaceutically acceptable excipient, or a mixture thereof. In some embodiments, the compositions of the present disclosure are formulated for use as a vaccine. In some embodiments, the compositions of the present application are formulated for use as an adjuvant.

[0154] Accordingly, in one aspect, provided herein are pharmaceutical compositions including a pharmaceutically acceptable excipient and: (a) a nucleic acid construct of the disclosure, and/or b) a recombinant cell of the disclosure.

[0155] Non-limiting exemplary embodiments of the pharmaceutical compositions of the disclosure can include one or more of the following features. In some embodiments, provided herein are compositions including a nucleic acid construct as disclosed herein and a pharmaceutically acceptable excipient. In some embodiments, provided herein are compositions including a recombinant cell as disclosed herein and a pharmaceutically acceptable excipient.

[0156] In some embodiments, the nucleic acid constructs of the disclosure (e.g., vectors or srRNA molecules) can be used in a naked form or formulated with a delivery vehicle. Exemplary delivery vehicles suitable for the compositions and methods of the disclosure include, but are not limited to exosomes, liposomes (e.g., neutral or anionic liposomes), microspheres, immune stimulating complexes (ISCOMS), lipid-based nanoparticles (LNP), solid lipid nanoparticles (SLN), polyplexes, polymer nanoparticles, viral replicon particles (VRP), or conjugated with bioactive ligands, which can facilitate delivery and/or enhance the immune response against EBV. These compounds are readily available to one skilled in the art; for example, see *Liposomes: A Practical Approach*, RCP New Ed, IRL Press (1990) and Mendes B.B. et al. Nanodelivery of nucleic acids, *Nat. Rev. Methods Primers* 2, 24, (2022). Accordingly, in some embodiments of the disclosure, the compositions of the disclosure that formulated in a liposome. Liposomes are enclosed nanoparticles with a bilayer membrane which show excellent performance in delivery of nucleic acid drugs (e.g., DNAs and RNAs such as srRNA) as well as different drug types. More information in this regard can be found in, for example, Hsairat H. et al, *OpenNano*, Vol. 11, May 2023 and Gao Y. et al. *Pharmaceutics*, 15(1):178, Jan 2023. Adjuvants other than liposomes and

the like are also used and are known in the art. A skilled artisan will appreciate that adjuvants may protect the antigen (*e.g.*, nucleic acid constructs, vectors, srRNA molecules) from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system.

[0157] The composition of the disclosure can be formulated in a format to be compatible with its intended route of administration, such as an exosome, liposome, lipid-based nanoparticle (LNP), polymer nanoparticle, polyplex, viral replicon particle (VRP), microsphere, immune stimulating complex (ISCOM), conjugate of bioactive ligand, or a combination of any thereof. For example, in some embodiments, the compositions of the disclosure that formulated in a liposome. In some embodiments, the compositions of the disclosure that formulated in a lipid-based nanoparticle (LNP). In some embodiments, the compositions of the disclosure that formulated in a polymer nanoparticle. In some embodiments, the compositions are immunogenic compositions, *e.g.*, composition that can stimulate an immune response against EBV in a subject. In some embodiments, the pharmaceutical compositions are formulated as an adjuvant. In some embodiments, the immunogenic compositions are formulated as a vaccine. The term “vaccine” as used herein refers to prophylactically or therapeutically immunize an individual against EBV. The vaccine according to the present disclosure immunizes an individual against EBV infection and EBV-associated diseases. Immunization relates to the process of stimulating and sensitizing the immune system towards the antigen(s) within the vaccine. According to the disclosure, prophylactic immunization refers to the first exposure of an individual's immune system, *e.g.*, a naïve immune system, to EBV antigens. Said first exposure results in the clearance of said antigens from the body of the exposed individual and in the development of EBV-antigen specific CD4⁺- and CD8⁺-cells and antibody-producing memory B-cells. Upon a second exposure, the immune system is able to prevent EBV infection and/or clear said infection more effectively thereby preventing or mitigating the development of EBV-associated diseases. Specifically, the effects of said prophylactic immunization manifest itself in at least one of the following: preventing infection of the immunized individual with EBV, modifying, or limiting the infection, aiding, improving, enhancing, or stimulating the recovery of said individual from infection and generating immunological memory that will prevent or limit a subsequent EBV infection. The presence of any of said effects can be tested for and detected by routine methods known to the person skilled in the

art. For example, the individual is challenged with one or more EBV antigens which have been part of the vaccine used and antibody titers and the number of T-cells against said one or more antigens are determined. Also, the induction of neutralizing antibodies that inhibit infection of human B-cells *in vitro* can be determined.

[0158] While equally provoking an immune response against EBV antigens, therapeutic immunization in accordance with the present disclosure can be performed on individuals that have been exposed to EBV prior to said immunization, *e.g.*, they are already infected with EBV. In this case, immunization leads to the reactivation of resting T effector cells, which are confronted with the cognate antigens in a form that these antigens are presented by professional antigen-presenting cells in association with MHC class I and/or MHC class molecules. Therapeutic immunization against EBV may prove particularly relevant in cases where the reactivation of the virus is undesirable such as, *e.g.* In transplant recipients or otherwise immunocompromised patients (*e.g.*, HIV-positive individuals, cancer patients, patients with severe inflammatory or autoimmune diseases), or in cases where EBV-reactivation can lead to or has led to the development of a disease like Non-Hodgkin lymphoma and posttransplant lymphoproliferative disorders (PTLD), oral hairy leukoplakia, chronic active EBV infection (CAEBV), or in cases where the B-cell transforming capacity of EBV has led to the development of a disease such as, *e.g.*, cancer.

[0159] In some embodiments, the methods of the disclosure include administration of srRNA-based pharmaceutical compositions as described herein against EBV infection that elicit potent neutralizing antibodies and robust T cell responses against EBV antigens, inhibit the production of viral immunomodulatory factors, and/or prevent viral latency.

[0160] In some embodiments, the srRNA-based pharmaceutical compositions (*e.g.*, vaccines) disclosed herein are administered therapeutically, *e.g.*, following infection with EBV (to treat the infection). In some embodiments, the vaccines of the present disclosure can be administered prophylactically to prevent or reduce the frequency of Hodgkin's lymphoma, Burkitt's lymphoma, gastric carcinoma, nasopharyngeal carcinoma, post-transplant lymphoproliferative disease, diffuse B cell lymphoma, and/or NK/T cell lymphoma.

[0161] As described in greater detail below, in some embodiments of the disclosure, the compositions as disclosed herein can be formulated with a delivery vehicle into a delivery system, wherein the delivery system can include one or more of the following: a physiologic buffer, a

liposome, a viral replicon particle (VRP), a lipid-based nanoparticle (LNP), a polymer nanoparticle, a physiologic buffer, a microsphere, an immune stimulating complex (ISCOM), a conjugate of bioactive ligand, and a combination of any thereof.

[0162] In some embodiments, the nucleic acid constructs of the disclosure can be delivered to a cell or a subject by a lipid-based nanoparticle (LNP). LNP are generally less immunogenic than viral particles. While many humans have preexisting immunity to viral particles there is no pre-existing immunity to LNP. In addition, adaptive immune response against LNP is unlikely to occur which enables repeat dosing of LNP.

[0163] The lipids suitable for the compositions and methods described herein can be cationic lipids, ionizable cationic lipids, anionic lipids, or neutral lipids.

[0164] In some embodiments, the LNP of the disclosure can include one or more ionizable lipids. As used herein, the term "ionizable lipid" refers to a lipid that is cationic or becomes ionizable (protonated) as the pH is lowered below the pKa of the ionizable group of the lipid, but is more neutral at higher pH values. At pH values below the pKa, the lipid is then able to associate with negatively charged nucleic acids (*e.g.*, oligonucleotides). As used herein, the term "ionizable lipid" includes lipids that assume a positive charge on pH decrease from physiological pH, and any of a number of lipid species that carry a net positive charge at a selective pH, such as physiological pH. Permanently cationic lipids such as DOTMA have proven too toxic for clinical use. The ionizable lipid can be present in lipid formulations according to other embodiments, preferably in a ratio of about 30 to about 70 Mol%, in some embodiments, about 30 Mol%, in other embodiments, about 40 Mol%, in other embodiments, about 45 Mol% in other embodiments, about 47.5 Mol% in other embodiments, about 50 Mol%, in still other embodiments, and about 60 Mol% in yet others ("Mol%" means the percentage of the total moles that is of a particular component). The term "about" in this paragraph signifies a plus or minus range of 5 Mol%. DODMA, or 1,2-dioleoyloxy-3-dimethylaminopropane, is an ionizable lipid, as is Dlin-MC3-DMA or 0-(Z,Z,Z,Z-heptatriaconta-6,9,26,29-tetraen-19-yl)-4-(N,N-dimethylamino) ("MC3").

[0165] In some embodiments, the LNP of the disclosure can include one or more ionizable cationic lipids. Several suitable ionizable cationic lipids have been developed for use in manufacturing of LNP. These include C12-200, MC3, LN16, and MD1 among others. Any of these cationic lipids can be used to formulate LNP for delivery of the nucleic acid constructs of the

disclosure. For example, in one type of LNP of the disclosure, a GalNAc moiety can be attached to the outside of the LNP and acts as a ligand for uptake into the liver via the asialoglycoprotein receptor.

[0166] In some embodiments, LNPs can be manufactured from cationic, anionic, or neutral lipids. Neutral lipids, such as the fusogenic phospholipid DOPE or the membrane component cholesterol, can be included in LNPs as 'helper lipids' to enhance transfection activity and nanoparticle stability. This is because in some instances, limitations of cationic lipids include low efficacy owing to poor stability and rapid clearance, as well as the generation of inflammatory or anti-inflammatory responses. LNPs can also have hydrophobic lipids, hydrophilic lipids, or both hydrophobic and hydrophilic lipids.

[0167] In some embodiments, the lipids or combination of lipids that are known in the art can be suitably used to produce LNPs include DOTMA, DOSPA, DOTAP, DMRIE, DC-cholesterol, DOTAP-cholesterol, GAP-DMORIE-DPyPE, and GL67A-DOPE-DMPE-polyethylene glycol (PEG). Non-limiting examples of suitable cationic lipids include 98N12-5, C12-200, DLin-KC2-DMA (KC2), DLin-MC3-DMA (MC3), XTC, MD1, and 7C1. Non-limiting examples of suitable neutral lipids include DPSC, DPPC, POPC, DOPE, and SM. Non-limiting examples of suitable PEG-modified lipids include PEG-DMG, PEG-CerC14, and PEG-CerC20.

[0168] In some embodiments, the lipids can be combined in any number of molar ratios to produce LNPs of the disclosure. In addition, the polynucleotide(s) can be combined with lipid(s) in a wide range of molar ratios to produce LNPs of the disclosure. For example, in some embodiments, the lipid of the LNP delivery system is present in mass ratio of lipid to RNA from about 100:1 to about 4:1. In some embodiments, the delivery system includes lipid-based nanoparticles having an average diameter of less than 1000 nm, less than 500 nm, less than 250 nm, less than 200 nm, less than 150 nm, less than 100 nm, less than 75 nm, less than 50 nm, or less than 25 nm. In some embodiments, the LNP delivery system includes lipid-based nanoparticles having an average diameter of about 1-1000 nm, about 1-1000 nm, about 1-500 nm, about 1-250 nm, about 25-200 nm, about 25-100 nm, about 35-75 nm, or about 25-60 nm.

[0169] In some embodiments, the pharmaceutical compositions are formulated for one or more of the following administration routes: intranasal administration, transdermal administration, intrathecal 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. In some particular embodiments of the disclosure, the composition is formulated intramuscular administration.

[0170] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.), or phosphate buffered saline (PBS). In these cases, the composition should be sterile and should be fluid to the extent that easy syringeability and/or injectability exists. It can be stable under the conditions of manufacture and storage, and can be preserved against the contaminating action of microorganisms such as bacteria and fungi. In the compositions of the disclosure, the carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, *e.g.*, sodium dodecyl sulfate. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be generally to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and/or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0171] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above.

[0172] In some embodiments, the pharmaceutical compositions of the disclosure, *e.g.*,

vaccine compositions, are formulated for inhalation, such as an aerosol, spray, mist, liquid, or powder. Administration by inhalation may be in the form of either dry powders or aerosol formulations, which are inhaled by a subject (*e.g.*, a patient) either through use of an inhalation device, *e.g.*, a microspray, a pressurized metered dose inhaler, or nebulizer.

[0173] In some embodiments, the composition is formulated for one or more of the following administration routes: intranasal administration, intrathecal administration, transdermal administration, intramuscular administration, intranodal administration, intravenous administration, intraperitoneal administration, oral administration, intravaginal, and intra-cranial administration. In some embodiments, the administered composition results in an increased production of interferon in the subject. In some embodiments, the administered composition induces production of one or more pro-inflammatory molecules in the subject. In some embodiments, the one or more pro-inflammatory molecules includes interferon alpha (IFN α), interferon gamma (IFN γ), TNF- α , IL-12, granzyme B, perforin, or a combination of any thereof.

METHODS OF THE DISCLOSURE

[0174] Administration of any one of the therapeutic compositions described herein, *e.g.*, nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions, can be used in the treatment of relevant health conditions associated with EBV infection, such as proliferative disorders (*e.g.*, cancers), infectious diseases (*e.g.*, infectious mononucleosis, acute infections, or chronic infections), and/or autoimmune diseases, and/or inflammatory diseases. In some embodiments, the nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions as described herein can be useful for modulating, *e.g.*, eliciting or suppressing a pharmacodynamic effect in a subject. Non-limiting examples of pharmacodynamic effect include immunogenicity effects, biomarker responses, therapeutic effects, prophylactic effects, desired effects, undesired effects, adverse effects, and effects in a disease or disease model. Accordingly, one aspect of the disclosure relates to methods for modulating a pharmacodynamic effect in a subject in need thereof, the methods include administering to the subject a composition including one or more of the following: (a) a nucleic acid construct as described herein (for example a replicon, *e.g.*, self-replicating RNA construct), (b) a recombinant cell as described herein; and (c) a pharmaceutical composition as described herein. In some embodiments, the pharmacodynamic effect includes 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. In some embodiments, the pharmacodynamic effect includes preventing viral infection. In some embodiments, the pharmacodynamic effect includes decreasing viral load in the subject. In some embodiments, the pharmacodynamic effect includes eliciting an immune response in the subject. A skilled artisan will appreciate that various aspects of the immune response can be elicited in the methods of disclosure. For example, in some embodiments, the elicited immune response includes an anti-EBV antibody response, a neutralizing antibody response, and an anti-EBV T cell response, or a combination of any thereof.

[0175] In some embodiments, the nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions as described herein can be incorporated into therapeutic agents for use in methods of treating a subject who has, who is suspected of having, or who may be at high risk for developing one or more relevant health conditions or diseases associated with EBV (e.g., inflammatory diseases, autoimmune disease, or cancers). Accordingly, in another aspect, provided herein are methods for preventing or treating a health condition in a subject, the methods include prophylactically or therapeutically administering to the subject a composition including one or more of the following: (a) a nucleic acid construct as described herein (for example a replicon, e.g., self-replicating RNA construct; (b) a recombinant cell as described herein; and (c) a pharmaceutical composition as described herein. In some embodiments, the administered composition elicits an immune response in the subject. In some embodiments, the administered composition induces production of one or more pro-inflammatory molecules in the subject. In some embodiments, the one or more pro-inflammatory molecules includes interferon alpha ($\text{IFN}\alpha$), interferon gamma ($\text{IFN}\gamma$), $\text{TNF-}\alpha$, IL-12, granzyme B, perforin, or a combination of any thereof. In some embodiments, the administered composition confers (e.g., results in) an increased production of interferon in the subject. In some embodiments, the subject is having or suspected of having an EBV-associated disease. In some embodiments, 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.

[0176] Exemplary health conditions or diseases can include, without limitation, cancers, rare diseases, immune diseases, autoimmune diseases, acute infections, and chronic infections inflammatory diseases such as infectious mononucleosis (IM), characterized by the abnormal

proliferation of EBV-infected B cells that results in massive activation and proliferation of immune cells, leading to lymphoid hyperplasia, tonsillitis, lymphadenopathy, and hepatosplenomegaly. In some embodiments, the subject is a patient under the care of a physician.

[0177] Examples of autoimmune and inflammatory diseases suitable for the methods of the disclosure include, but are not limited to, rheumatoid arthritis, osteoarthritis, Still's disease, systemic sclerosis, multiple sclerosis, ankylosing spondylitis, systemic lupus erythematosus, Sjogren's syndrome, diabetic retinopathy, diabetic vasculopathy, diabetic neuralgia, insulinitis, psoriasis, alopecia areata, warm and cold autoimmune hemolytic anemia (AIHA), pernicious anemia, acute inflammatory diseases, autoimmune adrenalitis, chronic inflammatory demyelinating polyneuropathy (CIDP), Lambert-Eaton syndrome, lichen sclerosis, Graves disease, Behçet's disease, Ménière's disease, reactive arthritis (Reiter's syndrome), Churg-Strauss syndrome, Cogan syndrome, CREST syndrome, pemphigus vulgaris and pemphigus foliaceus, bullous pemphigoid, polymyalgia rheumatica, polymyositis, primary biliary cirrhosis, pancreatitis, peritonitis, psoriatic arthritis, rheumatic fever, sarcoidosis, Sjörgensen syndrome, scleroderma, celiac disease, stiff-man syndrome, Takayasu arteritis, transient gluten intolerance, autoimmune uveitis, vitiligo, polychondritis, dermatitis herpetiformis (DH) or Duhring's disease, fibromyalgia, Goodpasture syndrome, Guillain-Barré syndrome, Hashimoto's thyroiditis, autoimmune hepatitis, inflammatory bowel disease (IBD), Crohn's disease, colitis ulcerosa, myasthenia gravis, immune complex disorders, glomerulonephritis, polyarteritis nodosa, anti-phospholipid syndrome, polyglandular autoimmune syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura (ITP), urticaria, autoimmune infertility, juvenile rheumatoid arthritis, sarcoidosis, and autoimmune cardiomyopathy.

[0178] Non-limiting examples of inflammatory diseases that can be suitably treated or prevented in the methods of the disclosure include inflammatory diseases such as asthma, inflammatory bowel disease (IBD), chronic colitis, splenomegaly, rheumatoid arthritis, polymyositis, and myocarditis. In some embodiments, the inflammatory disease is infectious mononucleosis (IM), characterized by the abnormal proliferation of EBV-infected B cells that results in massive activation and proliferation of immune cells, leading to lymphoid hyperplasia, tonsillitis, lymphadenopathy, and hepatosplenomegaly.

[0179] In some embodiments, the health condition is a proliferative disorder or a viral

infection (*e.g.*, EBV infection). In some embodiments, the subject has or is suspected of having a condition associated with proliferative disorder or a viral infection. In some embodiments, the health condition is a proliferative disorder is a lymphoproliferative disease or cancer associate with EBV infection, such as, posttransplant lymphoproliferative disease (PTLD), Hodgkin's lymphoma, Burkitt lymphoma, and nasopharyngeal cancer.

[0180] In some embodiments, the health condition is a rare disease, *e.g.*, a disease or condition that affects less than 200,000 people in the United States, as defined by The Orphan Drug Act (www.fda.gov/patients/rare-diseases-fda) and/or an inflammatory and/or autoimmune disorder. In some embodiments, the subject has or is suspected of having a condition associated with an inflammatory and/or autoimmune disorder and/or a rare disease (*e.g.* including but not limited to chronic active Epstein-Barr virus (CAEBV) disease).

[0181] In some embodiments, the disclosed composition is formulated to be compatible with its intended route of administration. For example, the nucleic acid constructs (*e.g.*, srRNA constructs), recombinant cells, and/or pharmaceutical compositions of the disclosure may be given orally or by inhalation, but it is more likely that they will be administered through a parenteral route. Examples of parenteral routes of administration include, for example, intramuscular, intratumoral, intraocular, intravenous, intranodal, intradermal, subcutaneous, transdermal (topical), transmucosal, intravaginal, and rectal administration. In some embodiments, the composition is administered intramuscularly. In some embodiments, the composition is administered intratumorally. Solutions or suspensions used for parenteral application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates, phosphates, tris, sucrose and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as mono- and/or di-basic sodium phosphate, hydrochloric acid or sodium hydroxide (*e.g.*, to a pH of about 7.2-7.8, *e.g.*, 7.5). The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0182] Dosage, toxicity and therapeutic efficacy of such subject nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions of the disclosure can be determined by

standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit high therapeutic indices are generally suitable. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0183] For example, the data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies generally within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the disclosure, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*e.g.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0184] The therapeutic compositions described herein, *e.g.*, nucleic acid constructs, *e.g.*, srRNA constructs, recombinant cells, and/or pharmaceutical compositions, can be administered one or more times. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the pharmaceutical compositions of the disclosure can include a single treatment or can include a series of treatments. In some embodiments, the compositions are administered one or more times, followed by a rest period of 2 to 56 days, *e.g.*, 28 days, followed by additional administration every 2-12 months. Repeated administration can be performed every 1-10 years. With regard to nucleic acid constructs (*e.g.*, srRNA constructs), the therapeutically effective amount of a nucleic

acid construct of the disclosure (*e.g.*, an effective dosage) depends on the nucleic acid construct selected. For instance, single dose amounts in the range of approximately 0.001 to 0.1 mg/kg of patient body weight can be administered. In some embodiments, about 0.005, 0.01, 0.05 mg/kg may be administered. In some embodiments, single dose amounts in the range of approximately 0.001 µg to 300 µg/kg of patient body weight can be administered. In some embodiments, single dose amounts in the range of approximately 0.3 mg to 3 mg/kg of patient body weight can be administered.

[0185] As discussed *supra*, a therapeutically effective amount includes an amount of a therapeutic composition that is sufficient to promote a particular effect when administered to a subject, such as one who has, is suspected of having, or is at risk for a health condition, *e.g.*, a disease or infection. In some embodiments, an effective amount includes an amount sufficient to prevent or delay the development of a symptom of the disease or infection, alter the course of a symptom of the disease or infection (for example but not limited to, slow the progression of a symptom of the disease or infection), or reverse a symptom of the disease or infection. It is understood that for any given case, an appropriate effective amount can be determined by one of ordinary skill in the art using routine experimentation.

[0186] The efficacy of a treatment including a disclosed therapeutic composition for the treatment of disease or infection can be determined by the skilled clinician. However, a treatment is considered effective treatment if at least any one or all of the signs or symptoms of disease or infection are improved or ameliorated. Efficacy can also be measured by failure of an individual to worsen as assessed by hospitalization or need for medical interventions (*e.g.*, progression of the disease or infection is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Treatment includes any treatment of a disease or infection in a subject or an animal (some non-limiting examples include a human, or a mammal) and includes: (1) inhibiting the disease or infection, *e.g.*, arresting, or slowing the progression of symptoms; or (2) relieving the disease or infection, *e.g.*, causing regression of symptoms; and (3) preventing or reducing the likelihood of the development of symptoms.

[0187] In some embodiments, the nucleic acid constructs (*e.g.*, srRNA constructs), recombinant cells, and/or pharmaceutical compositions of the disclosure can be administered to a subject in a composition having a pharmaceutically acceptable carrier and in an amount effective

to stimulate an immune response. Generally, a subject can be immunized through an initial series of injections (or administration through one of the other routes described below) and subsequently given boosters to increase the protection afforded by the original series of administrations. The initial series of injections and the subsequent boosters are administered in such doses and over such a period of time as is necessary to stimulate an immune response in a subject. In some embodiments, the administered composition results in an increased production of interferon in the subject. In some embodiments of the disclosed methods, the subject is a mammal. In some embodiments, the mammal is a human subject.

[0188] As described above, pharmaceutically acceptable carriers suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In these cases, the composition must be sterile and must be fluid to the extent that easy syringeability exists. The composition must further be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, *etc.*), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like.

[0189] Sterile injectable solutions can be prepared by incorporating the nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[0190] When the nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions are suitably protected, as described above, they may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the

individual's diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

[0191] As discussed *supra*, in some embodiments of the disclosure, the compositions as disclosed herein, *e.g.*, nucleic acid constructs of the disclosure, can be formulated with a delivery vehicle into a delivery system, wherein the delivery system can include one or more of the following: a physiologic buffer, a liposome, a viral replicon particle (VRP), a lipid-based nanoparticle (LNP), a polymer nanoparticle, a physiologic buffer, a microsphere, an immune stimulating complex (ISCOM), a conjugate of bioactive ligand, and a combination of any thereof.

[0192] In some embodiments, the nucleic acid constructs of the disclosure can be delivered to a cell or a subject by a lipid-based nanoparticle (LNP). LNP are generally less immunogenic than viral particles. While many humans have preexisting immunity to viral particles there is no pre-existing immunity to LNP. In addition, adaptive immune response against LNP is unlikely to occur which enables repeat dosing of LNP.

[0193] In some embodiments, the therapeutic compositions described herein, *e.g.*, nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions are incorporated into therapeutic compositions for use in methods of preventing or treating a subject who has, who is suspected of having, or who may be at high risk for developing a cancer, an autoimmune disease, an inflammatory disease, and/or an infection. In some embodiments, the microbial infection is a viral infection, *e.g.*, EBV infection.

Additional therapies

[0194] In some embodiments, a composition according to the present disclosure is administered to the subject individually as a single therapy (monotherapy) or as a first therapy in combination with at least one additional therapies (*e.g.*, second therapy). In some embodiments, the second therapy is selected from the group consisting of chemotherapy, radiotherapy, immunotherapy, hormonal therapy, toxin therapy, targeted therapy, and surgery. In some embodiments, the second therapy is selected from the group consisting of chemotherapy, radiotherapy, immunotherapy, hormonal therapy, toxin therapy or surgery. In some embodiments, the first therapy and the second therapy are administered concomitantly. In some embodiments, the first therapy is administered at the same time as the second therapy. In some embodiments, the first

therapy and the second therapy are administered sequentially. In some embodiments, the first therapy is administered before the second therapy. In some embodiments, the first therapy is administered after the second therapy. In some embodiments, the first therapy is administered before and/or after the second therapy. In some embodiments, the first therapy and the second therapy are administered in rotation. In some embodiments, the first therapy and the second therapy are administered together in a single formulation.

KITS

[0195] Also provided herein are various kits for the practice of a method described herein as well as written instructions for making and using the same. In particular, some embodiments of the disclosure provide kits for eliciting a pharmacodynamic effect. Some embodiments of the disclosure provide kits for eliciting an immune response in a subject. Some other embodiments relate to kits for the prevention of a health condition in a subject in need thereof. Some other embodiments relate to kits for methods of treating a health condition in a subject in need thereof. For example, in some embodiments, the present disclosure provides kits that include one or more of the nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions as provided and described herein, as well as written instructions for making and using the same. In some embodiments, the kits are for the prevention and/or treatment of one or more EBV-associated diseases as described above. In some particular embodiments, the EBV-associated diseases include infectious mononucleosis (IM), autoimmune and/or inflammatory disease, and/or a rare disease, and/or cancer.

[0196] In some embodiments, the kits of the disclosure further include one or more means useful for the administration of any one of the provided nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions to a subject. For example, in some embodiments, the kits of the disclosure further include one or more syringes (including pre-filled syringes) and/or catheters (including pre-filled syringes) used to administer any one of the provided nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions to a subject. In some embodiments, a kit can have one or more additional therapeutic agents that can be administered simultaneously or sequentially with the other kit components for a desired purpose, *e.g.*, for eliciting an immune response, preventing, and/or treating a health condition in a subject in need thereof.

[0197] Any of the above-described kits can further include one or more additional reagents,

where such additional reagents can be selected from: dilution buffers, reconstitution solutions, wash buffers, control reagents, control expression vectors, negative controls, positive controls, reagents suitable for *in vitro* production and/or administration of the provided nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions of the disclosure.

[0198] In some embodiments, the components of a kit can be in separate containers. In some other embodiments, the components of a kit can be combined in a single container. Accordingly, in some embodiments of the disclosure, the kit includes one or more of the nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions as provided and described herein in one container (*e.g.*, in a sterile glass or plastic vial) and a further therapeutic agent in another container (*e.g.*, in a sterile glass or plastic vial).

[0199] In another embodiment, the kit includes a combination of the compositions described herein, including one or more nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions of the disclosure in combination with one or more further therapeutic agents formulated together, optionally, in a pharmaceutical formulation, in a single, common container.

[0200] In some embodiments wherein the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can include a device (*e.g.*, an injection device or catheter) for performing such administration. For example, the kit can include one or more hypodermic needles or other injection devices as discussed above containing one or more nucleic acid constructs, recombinant cells, and/or pharmaceutical compositions of the disclosure.

[0201] In some embodiments, a kit can further include instructions for using the components of the kit to practice one or more of the methods disclosed herein. For example, the kit can include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids patients and physicians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the disclosure may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and intellectual property information.

[0202] The instructions for practicing the methods are generally recorded on a suitable

recording medium. For example, the instructions can be printed on a substrate, such as paper or plastic, *etc.* The instructions can be present in the kit as a package insert, in the labeling of the container of the kit or components thereof (*e.g.*, associated with the packaging or sub-packaging), *etc.* The instructions can be present as an electronic storage data file present on a suitable computer readable storage medium, *e.g.* CD-ROM, diskette, flash drive, *etc.* In some instances, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source (*e.g.*, via the internet), can be provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions can be recorded on a suitable substrate or recording medium.

[0203] All publications and patent applications mentioned in this disclosure are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0204] No admission is made that any reference cited herein constitutes prior art. The discussion of the references states what their authors assert, and the Applicant reserves the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that, although a number of information sources, including scientific journal articles, patent documents, and textbooks, are referred to herein; this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0205] The discussion of the general methods given herein is intended for illustrative purposes only. Other alternative methods and alternatives will be apparent to those of skill in the art upon review of this disclosure, and are to be included within the spirit and purview of this application.

[0206] Additional embodiments are disclosed in further detail in the following examples, which are provided by way of illustration and are not in any way intended to limit the scope of this disclosure or the claims.

EXAMPLES

[0207] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, cell biology, biochemistry, nucleic acid chemistry, and immunology, which are well known to those skilled in the art. Such techniques

are explained fully in the literature, such as Sambrook, J., & Russell, D. W. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory and Sambrook, J., & Russel, D. W. (2001). *Molecular Cloning: A Laboratory Manual* (3rd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory (jointly referred to herein as “Sambrook”); Ausubel, F. M. (1987). *Current Protocols in Molecular Biology*. New York, NY: Wiley (including supplements through 2014); Bollag, D. M. *et al.* (1996). *Protein Methods*. New York, NY: Wiley-Liss; Huang, L. *et al.* (2005). *Nonviral Vectors for Gene Therapy*. San Diego: Academic Press; Kaplitt, M. G. *et al.* (1995). *Viral Vectors: Gene Therapy and Neuroscience Applications*. San Diego, CA: Academic Press; Lefkovits, I. (1997). *The Immunology Methods Manual: The Comprehensive Sourcebook of Techniques*. San Diego, CA: Academic Press; Doyle, A. *et al.* (1998). *Cell and Tissue Culture: Laboratory Procedures in Biotechnology*. New York, NY: Wiley; Mullis, K. B., Ferré, F. & Gibbs, R. (1994). *PCR: The Polymerase Chain Reaction*. Boston: Birkhauser Publisher; Greenfield, E. A. (2014). *Antibodies: A Laboratory Manual* (2nd ed.). New York, NY: Cold Spring Harbor Laboratory Press; Beaucage, S. L. *et al.* (2000). *Current Protocols in Nucleic Acid Chemistry*. New York, NY: Wiley, (including supplements through 2014); and Makrides, S. C. (2003). *Gene Transfer and Expression in Mammalian Cells*. Amsterdam, NL: Elsevier Sciences B.V., the disclosures of which are incorporated herein by reference.

[0208] Additional embodiments are disclosed in further detail in the following examples, which are provided by way of illustration and are not in any way intended to limit the scope of this disclosure or the claims.

EXAMPLE 1

Construction of modified alphavirus vectors

[0209] This Example describes experiments performed to construct a base alphavirus vector (*e.g.*, without a heterologous gene) that are subsequently used for construction of alphavirus vectors encoding polypeptide constructs of interest, *e.g.*, polypeptide constructs comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV).

[0210] The base VEEV vector was synthesized *de novo* in four ~4 kb parts (Twist Bioscience, Thermo Fisher GeneArt) from a TC-83 strain reference sequence (Genbank L01443) with a silent A2087G mutation, and a unique *SpeI* restriction enzyme cut site in place of the coding

sequence of the VEEV structural genes (where the 5' A is the next nucleotide after a P2A sequence following nucleotide 93 of the structural polyprotein gene, and the 3' T matches the location of the structural polyprotein's stop codon TGA). A 5' adaptor sequence (5'-CTGGAGACGTGGAGGAGAACCCTGGACCT-3'; SEQ ID NO: 59) was inserted upstream of the *SpeI* site, and a 3' adaptor sequence (5'-GACCGCTACGCCCCAATGACCCGACCAGC-3'; SEQ ID NO: 60) was inserted downstream of the *SpeI* site for subsequent Gibson Assembly® procedures. A bacteriophage T7 RNA polymerase promoter (5'-TAATACGACTCACTATAG-3'; SEQ ID NO: 61) was included upstream of the VEEV genome sequence, and downstream contained a poly(A) sequence followed by a *SapI* site, which cuts upstream of the recognition site. Immediately downstream of the *SapI* site is a T7 terminator sequence (5'-AACCCCTCTCTAAACGGAGGGGTTTTTTT-3'; SEQ ID NO: 62) followed by a unique *NotI* restriction enzyme cut site. The parts were combined in a five-piece Gibson Assembly® reaction (e.g., a linearized pYL backbone and the four synthesized fragments) to result in the VEEV base vector.

[0211] The EBV genes were codon optimized for human expression *in silico* (IDT) and synthesized *de novo*. The synthetic products were amplified using primers which added either 5' and 3' adaptor sequences to the ends of the genes, or primers which added sequences of homology to neighboring gene inserts. The *SpeI*-linearized base vector and the PCR products were combined by Gibson Assembly® procedure to result in the final vectors. Schematic depictions of twenty-two (22) exemplary nucleic acid constructs expressing EBV antigenic determinants derived from one or more EBV genes are shown in **FIG. 2**. In each construct, the numbers of EBV antigens and their ordinality in the antigen cassette are shown. Some constructs contain coding sequences for multiple polypeptides operably linked to one another within a single polycistronic ORF (see, e.g., Rep-674, Rep-675, Rep-678, Rep-679, Rep-680, Rep 728, Rep-729, Rep-730, and Rep-731). Some other constructs include coding sequences for multiple polypeptides configured into two ORFs that are connected to one another by a sequence for an internal ribosomal entry site (IRES) from ECMV (see, e.g., Rep-692, Rep-693, Rep-694, Rep-696, Rep-697, Rep-698, Rep-699, Rep-733, Rep-734, Rep-735, Rep-736, and Rep-737). In **FIG. 2**, the EBV polypeptides, autoproteolytic peptide P2A sequences, and internal ribosomal entry sites (IRES) are shown in N-terminus to C-terminus direction (*i.e.*, in 5' to 3' direction) of the corresponding coding sequences.

EXAMPLE 2*In vitro* evaluation of srRNA constructs expressing selected EBV antigens

[0212] This Example describes *in vitro* experiments that were performed to evaluate expression levels and bioactivity of the synthetic srRNA constructs described in Example 1 above, and to investigate any differential behavior thereof (*e.g.*, replication and protein expression).

[0213] *In vitro* transcription: RNA was prepared by *in vitro* transcription from a *SapI*-linearized plasmid template with bacteriophage T7 RNA polymerase (HiScribe™ T7 High Yield RNA Synthesis Kit, NEB) followed by addition of a 5' cap 1 (Vaccinia Capping System, mRNA Cap 2'-O-Methyltransferase, NEB). RNA was purified using LiCl precipitation, followed by a 70% ethanol wash and resuspended in 1 mM sodium citrate pH 6.4. RNA concentration was determined by absorbance at 260 nm (Nanodrop, Thermo Fisher Scientific).

[0214] *Bioactivity (RNA replication)*: The srRNA-based bioactivity was measured by detection of the replication intermediate dsRNA in BHK-21 cells. RNA was transformed into cells by electroporation (*4D-Nucleofector*™, Lonza). At 15-22 hours following transformation, the cells were fixed and permeabilized (eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set, Invitrogen) and stained using a PE-conjugated anti-dsRNA mouse monoclonal antibody (J2, Scicons) to quantify the frequency of dsRNA+ cells by fluorescence flow cytometry. The frequency of dsRNA+ cells is shown plotted for each construct in **FIG. 3**, showing that the EBV srRNA vaccine constructs exhibit RNA replication.

[0215] *Protein expression (gp350 and gH/gL)*: In these experiments, protein expression was detected by flow cytometry using antibodies recognizing gp350 and the gH/gL complex. Mean fluorescence intensities (MFIs) of the fluorescence-labeled antibodies are plotted as a measure of protein expression. RNA was transformed by electroporation into BHK-21 cells (*e.g.*, *4D-Nucleofector*™, Lonza). At 15-22 hours following transformation, the cells were fixed and permeabilized (eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set, Invitrogen) and stained with an IgG2a anti-gp350 antibody, followed by an APC-conjugated anti-IgG2a secondary antibody, followed by a PE-Cy7-conjugated anti-gH/gL antibody. The MFI from gp350+ and gH/gL+ cells were measured by flow cytometry and the results are shown in **FIG. 4**. This figure shows relative expression from three out of five antigens in various multigenic or monogenic srRNA vaccine constructs in electroporated BHK-21 cells.

[0216] Protein expression (BZLF1): In these experiments, protein expression was detected by Western blotting using whole cell lysates from electroporated BHK-21 cells using an antibody to BZLF1. RNA was transformed by electroporation into BHK-21 cells (*e.g.*, 4D-Nucleofector™, Lonza) and collected for lysis at 15-22 hours following transformation. The protein content of the lysates was measured by bicinchoninic acid assay (BCA) and normalized before running the samples in duplicate on an electrophoretic gel. After transfer to a nitrocellulose membrane, the membrane was cut and probed with either an anti-BZLF1 antibody, or an anti-actin antibody. The results are shown in **FIG. 5** for various monogenic and multigenic srRNA vaccine constructs in electroporated BHK-21 cells. **FIG. 5** shows relative BZLF1 expression from the different constructs (left), controlled by the observed actin staining (right).

[0217] These data demonstrate that the EBV srRNA vaccine constructs undergo RNA replication (**FIG. 3**), and express the encoded gp350, gH/gL (**FIG. 4**), and BZLF1 (**FIG. 5**) antigens required to elicit an immune response.

EXAMPLE 3

srRNA constructs expressing selected EBV antigens elicit antibody response *in vivo*

[0218] This Example describes experiments that were performed to demonstrate that srRNA-based vaccines can generate antibody responses *in vivo* to encoded EBV antigens.

[0219] LNP formulation. srRNA was formulated in lipid nanoparticles using a microfluidics mixer and analyzed for particle size, polydispersity using dynamic light scattering and encapsulation efficiency is determined by a RiboGreen® (dye exclusion) assay. Lipids were suspended in ethanol. RNA was suspended in 50 mM citrate pH 4 at a concentration of 103 µg/ml, and was mixed at a flow rate ratio of 3:1 (aqueous:organic) with a total flow rate of 12 mL/min.

[0220] Mice and injections. CD1 ISG mice were purchased from Charles River Labs, Envigo, or Jackson Laboratories. On the day of dosing, 1 µg of material was injected intramuscularly either into one or split into both quadriceps muscles. Animals were dosed on Day 0 and Day 28. Animals were monitored for body weight and other general observations throughout the course of the study.

[0221] Antibody response. An IgG ELISA was used to quantify the *in vivo* antibody response to the surface EBV antigens gH/gL complex and gp350 encoded on various srRNA vaccine constructs. In these experiments, sera from immunized mice were collected for measurement 41

days after the first dose. A standard mouse antibody to each EBV antigen was used to quantify responses. **FIG. 6A** shows the results of anti-gH/gL IgG responses, and **FIG. 6B** shows the results of anti-gp350 IgG responses, a direct ELISA method to EBV proteins gp350 and gH/gL complex using sera from immunized mice.

[0222] These data demonstrate that srRNA-based vaccines can generate an immune response in the form of antibody responses to surface EBV antigens gH/gL complex and gp350 *in vivo*.

EXAMPLE 4

srRNA constructs expressing selected EBV antigens elicit T cell response *in vivo*

[0223] This Example describes experiments that were performed to demonstrate that srRNA-based vaccines can generate T cell responses *in vivo* to encoded EBV antigens.

[0224] LNP formulation. srRNA was formulated in lipid nanoparticles using a microfluidics mixer and analyzed for particle size, polydispersity using dynamic light scattering and encapsulation efficiency is determined by a RiboGreen® (dye exclusion) assay. Lipids were suspended in ethanol. RNA was suspended in 50 mM citrate pH 4 at a concentration of 103 µg/ml, and was mixed at a flow rate ratio of 3:1 (aqueous:organic) with a total flow rate of 12 mL/min.

[0225] Mice and injections. CD1 ISG mice were purchased from Charles River Labs, Envigo, or Jackson Laboratories. On the day of dosing, 1 µg of material was injected intramuscularly either into one or split into both quadricep muscles. Animals were dosed on Day 0 and Day 28. Animals were monitored for body weight and other general cageside observations throughout the course of the study. Serum was collected throughout the study for analysis of anti-EBV antibody responses. Spleens were collected at the end of the study (Day 41) to assess anti-EBV T cell responses.

[0226] ELISpot. To measure the magnitude of EBV antigen-specific T cell responses, splenocytes were stimulated with peptide pools corresponding to each encoded antigen and secretion of IFN γ was detected using a commercial ELISpot kit. T cell responses 41 days after the first dose are shown to five encoded antigens, gH/gL (**FIG. 7A**), gp350 (**FIG. 7B**), LMP2B (**FIG. 7C**), and BZLF1 (**FIG. 7D**). The total T cell responses (plotted as counted spot-forming units per million cells) are shown the y-axis.

[0227] These data demonstrate that srRNA-based EBV vaccines can generate T cell responses *in vivo* to encoded EBV antigens.

EXAMPLE 5

srRNA constructs expressing selected EBV antigens elicit neutralizing antibodies *in vivo*

[0228] This Example describes experiments that were performed to demonstrate that srRNA-based vaccines can generate neutralizing antibodies *in vivo* to EBV.

[0229] LNP formulation. srRNA was formulated in lipid nanoparticles using a microfluidics mixer and analyzed for particle size, polydispersity using dynamic light scattering and encapsulation efficiency is determined by a RiboGreen® (dye exclusion) assay. Lipids were suspended in ethanol. RNA was suspended in 50 mM citrate pH 4 at a concentration of 103 µg/ml, and was mixed at a flow rate ratio of 3:1 (aqueous:organic) with a total flow rate of 12 mL/min.

[0230] Mice and injections. CD1 ISG mice were purchased from Charles River Labs, Envigo, or Jackson Laboratories. On the day of dosing, vaccines were administered intramuscularly either into one or split into both quadricep muscles. Animals were dosed on Day 0, Day 56, and Day 120. Animals were monitored for body weight and other general cageside observations throughout the course of the study. Serum was collected throughout the study for analysis of anti-EBV antibody responses.

[0231] EBV infection neutralization. To measure the presence of antibodies that neutralize EBV infection, serum was collected from mice with 1, 2, or 3 administrations of srRNA vaccines and were evaluated in a viral neutralization assay using the Raji B cell line (**FIG. 8**). The levels of 50% neutralizing titer (NT50) are shown as bars representing the geometric means and error bars represent the geometric standard deviation. Kruskal-Wallis statistical comparisons are shown between the groups.

[0232] These data demonstrate that srRNA-based EBV vaccines can generate neutralizing antibody responses *in vivo* to EBV.

[0233] While particular alternatives of the present disclosure have been disclosed, it is to be understood that various modifications and combinations are possible and are contemplated within the true spirit and scope of the appended claims. There is no intention, therefore, of limitations to the exact abstract and disclosure herein presented.

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid construct comprising a nucleic acid sequence encoding a modified alphavirus genome or self-replicating RNA (srRNA), wherein at least a portion of the nucleic acid sequence encoding the viral structural proteins of the modified alphavirus genome or srRNA has been replaced by coding sequences for a polypeptide construct comprising one or more of antigenic determinants derived from Epstein-Barr virus (EBV).
2. The nucleic acid construct of claim 1, wherein one or more of antigenic determinants is derived from one or more EBV polypeptides/antigens encoded by a lytic gene, a pre-latent gene, a latent gene, an early gene, an immediate-early gene, a late gene, a reactivation from latency gene, a variant of any thereof, or a combination of any thereof.
3. The nucleic acid construct of any one of claims 1-2, wherein the EBV latent gene is selected from the group consisting of LMP2, BKRF1, BYRF1, BLRF3/BERF1, BERF2a/b, BERF3/4, BamHI-W, BNLF1 (LMP1), BARTs, and EBER1/2.
4. The nucleic acid construct of any one of claims 1-3, wherein the EBV latent gene is LMP2, BNLF1 (LMP1), BKRF1 (EBNA1), BLRF3/BERF1 (EBNA3A-C, -LP), and BYRF1 (EBNA2).
5. The nucleic acid construct of any one of claims 1-4, wherein the EBV immediate-early gene is selected from the group consisting of BZLF1, BRLF1, and BMLF1.
6. The nucleic acid construct of any one of claims 1-5, wherein the EBV early gene is selected from the group consisting of BRRF1, BORF2, BaRF1, BXLF1, BGLF5, BLLF3, BKRF3, BALF5, BMRF1, BALF2, BSLF1, BBLF2/3, BBLF4, BSLF2, BHRF1, BALF1, BARF1, BGLF4, BFRF1, BHLF1, BHLF2, and BNLF2a.
7. The nucleic acid construct of any one of claims 1-6, wherein the EBV late gene is selected from the group consisting of BLLF1 (gp350/220), BNRF1, BPLF1, BOLF1, BVRF1, BBLF1, BGLF1, BSRF1, BRRF2, BDLF2, BKRF4, BcLF1, BDLF1, BFRF3, BLRF2, BdRF1, BBRF1, BVRF2, BGLF2, BORF1, BLRF1 (gN), BZLF2, BKRF2 (gp25, gL), BBRF3 (gM), BXLF2 (gp85, gH), BILF1, BILF2, BALF4 (gp100, gB), BDLF3, BMRF2, BALF3, and BCRF1.

8. The nucleic acid construct of any one of claims 1-7, wherein the EBV lytic gene is selected from the group consisting of BLLF1 (gp350/220), BXLF2 (gp85, gH), BALF4 (gp100, gB), BSLF1 (gp42), and BKRF2 (gp25, gL).
9. The nucleic acid construct of any one of claims 1-8, wherein the EBV pre-latent gene is BYRF1 (EBNA2).
10. The nucleic acid construct of any one of claims 1-7, wherein the one or more EBV genes is selected from the group consisting of BLLF1 (gp350/220), BZLF1, BXLF2 (gp85, gH), BKRF2 (gp25, gL), LMP2, and a combination of any thereof.
11. The nucleic acid construct of any one of claims 1-10, wherein the coding sequences for the polypeptide construct comprises coding sequences for the following genes:
- (a) BZLF1 or a variant thereof;
 - (b) BLLF1 (gp350/220) or a variant thereof;
 - (c) LMP2B or a variant thereof;
 - (d) gH and gL, or a variant of any thereof;
 - (e) BZLF1, LMP2B, gH, gL, and BLLF1 (gp350/220), or a variant of any thereof;
 - (f) gH, gL, and BLLF1 (gp350/220), or a variant of any thereof; or
 - (g) BZLF1 and LMP2B, or a variant of any thereof.
12. The nucleic acid construct of any one of claims 1-11, wherein the BLLF1 (gp350/220) or variant thereof is devoid of a functional transmembrane (TM) domain.
13. The nucleic acid construct of any one of claims 1-12, wherein the gH or variant thereof is devoid of a functional transmembrane (TM) domain.
14. The nucleic acid construct of any one of claims 1-13, wherein the BZLF1 or variant thereof is devoid of (i) a functional nuclear localization sequence (NLS), and/or (ii) a functional transactivation (TA) domain.
15. The nucleic acid construct of any one of claims 1-14, wherein the polypeptide construct comprises an amino acid sequence having 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 an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-34.

16. The nucleic acid construct of any one of claims 1-15, wherein the coding sequences for the antigenic determinants are operably linked to one another within a single open reading frame (*i.e.*, in a polycistronic ORF).

17. The nucleic acid construct of any one of claims 1-15, wherein the coding sequences for the antigenic determinants are operably linked to one another within two or more ORFs.

18. The nucleic acid construct of any one of claims 1-17, wherein the coding sequences for the antigenic determinants are operably linked to one another by one or more linkers.

19. The nucleic acid construct of claim 18, wherein the one or more linkers comprises coding sequences for an autoproteolytic peptide sequence or an internal ribosomal entry site (IRES).

20. The nucleic acid construct of claim 19, wherein the autoproteolytic peptide sequence comprises one or more autoproteolytic cleavage sequences 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 Thossea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof.

21. The nucleic acid construct of claim 19, wherein the internal ribosomal entry site (IRES) is 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.

22. The nucleic acid construct of any one of claims 1-18, wherein the one or more of EBV antigenic determinants is of an EBV type 1 (EBV-1), or an EBV type 2 (EBV-2), or a combination

thereof.

23. The nucleic acid construct of any one of claims 1-22, wherein the one or more of EBV antigenic determinants is of a virulent EBV strain or an avirulent EBV strain.

24. The nucleic acid construct of any one of claims 22-23, wherein the one or more of EBV antigenic determinants is derived from an AG876 strain, an Akata strain, an Alaska strain, a B95-8 strain, a Hina 1 strain, a HKNPC strain, a China 2 strain, a CVI 988 strain, a GD1 strain, a GD2 strain, a GP202 strain, a M81 strain, a Mediterranean strain, a Mutu strain, a P3HR1 strain, a Raji strain, a SNU-719 strain, a YCCEL1 strain, or a combination thereof.

25. The nucleic acid construct of any one of claims 1-24, wherein the modified alphavirus genome or srRNA comprises no nucleic acid sequence encoding viral structural proteins.

26. The nucleic acid construct of any one of claims 1-25, wherein the nucleic acid sequence encoding the polypeptide construct is operably linked to a promoter sequence.

27. The nucleic acid construct of claim 26, wherein the promoter sequence is a 26S subgenomic (sg) promoter.

28. The nucleic acid construct of any one of claims 1-27, wherein the srRNA is a capped srRNA comprising a 5'-cap.

29. The nucleic acid construct of claim 28, wherein the capped srRNA is a co-transcriptionally capped srRNA.

30. The nucleic acid construct of claim 28, wherein the capped srRNA is an enzymatically capped srRNA.

31. The nucleic acid construct of any one of claims 1-30, wherein the modified alphavirus genome or srRNA is of an alphavirus belonging to the VEEV/EEEV group, or the SFV group, or the SINV group.

32. The nucleic acid of claim 31, wherein the alphavirus is Venezuelan equine encephalitis virus

(VEEV), Eastern equine encephalitis virus (EEEV), Chikungunya virus (CHIKV), Madariaga virus (MADV), Western equine encephalitis virus (WEEV), or Sindbis virus (SINV).

33. The nucleic acid construct of any one of claims 1-32, wherein 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 a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 35-56.

34. A recombinant cell comprising a nucleic acid construct according to any one of any one of claims 1-33.

35. The recombinant cell of claim 34, wherein the recombinant cell is a eukaryotic cell.

36. The recombinant cell of claim 34, wherein the recombinant cell is an animal cell.

37. The recombinant cell of claim 36, wherein the animal cell is a vertebrate animal cell or an invertebrate animal cell.

38. The recombinant cell of claim 37, wherein the recombinant cell is an insect cell.

39. The recombinant cell of claim 38, wherein the recombinant insect cell is a mosquito cell.

40. The recombinant cell of claim 37, wherein the recombinant cell is a mammalian cell.

41. The recombinant cell of claim 37, wherein the recombinant cell is selected from the group consisting of a monkey kidney CV1 cell transformed by SV40, a human embryonic kidney cell (HEK), a baby hamster kidney cell (BHK) or a derivative cell thereof, a mouse sertoli cell, a monkey kidney cell, a human cervical carcinoma cell, a canine kidney cell, a buffalo rat liver cell, a human lung cell, a human liver cell, a mouse mammary tumor, a TRI cell, a FS4 cell, a Chinese hamster ovary cell (CHO), an African green monkey kidney 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 B cell, a human epithelial cell, a human T cell, a human dendritic 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.

42. A cell culture comprising at least one recombinant cell according to any one of claims 34-41 and a culture medium.

43. A transgenic animal comprising:

- (a) a nucleic acid construct according to any one of claims 1-33; and/or
- (b) a recombinant cell according to any one of claims 34-41.

44. The transgenic animal of claim 43, wherein the animal is a vertebrate animal or an invertebrate animal.

45. The transgenic animal of claim 43, wherein the animal is an insect.

46. The transgenic animal of claim 43, wherein the animal is a mammalian.

47. The transgenic animal of claim 46, wherein the mammalian is a non-human mammalian.

48. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and:

- (a) a nucleic acid construct according to any one of claims 1-33; and/or
- (b) a recombinant cell according to any one of claims 34-41.

49. The pharmaceutical composition of claim 48, wherein the composition comprises a nucleic acid construct of any one of claims 1-33, and a pharmaceutically acceptable excipient.

50. The pharmaceutical composition of any one of claims 48-49, the composition is formulated with a delivery vehicle into a delivery system, wherein the delivery system comprises a liposome, a viral replicon particle (VRP), a lipid-based nanoparticle (LNP), a polymer nanoparticle, a physiologic buffer, a microsphere, an immune stimulating complex (ISCOM), a conjugate of bioactive ligand, or a combination of any thereof.

51. The pharmaceutical composition of claim 50, wherein the LNP delivery system comprises a cationic lipid, an ionizable cationic lipid, an anionic lipid, or a neutral lipid.

52. The pharmaceutical composition of claim 51, wherein the lipid of the LNP delivery system is present in mass ratio of lipid to RNA from about 100:1 to about 4:1.
53. The pharmaceutical composition of claim 52, wherein the LNP delivery system comprises lipid-based nanoparticles having an average diameter of less than 1000 nm, 500 nm, 250 nm, 200 nm, 150 nm, 100 nm, 75 nm, 50 nm, or 25 nm.
54. The pharmaceutical composition of claim 52, wherein the LNP delivery system comprises lipid-based nanoparticles having an average diameter of about 1 - 1000 nm, about 1 - 1000 nm, about 1 - 500 nm, about 1 - 250 nm, about 25 - 200 nm, about 25 - 100 nm, about 35 - 75 nm, or about 25 - 60 nm.
55. The pharmaceutical composition of any one of claims 48-54, wherein the composition is an immunogenic composition.
56. The pharmaceutical composition of claim 55, wherein the composition is formulated as a vaccine or an adjuvant.
57. The pharmaceutical composition of any one of claims 48-56, wherein the composition is formulated for one or more of the following administration routes: 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.
58. The pharmaceutical composition of claim 57, wherein the composition is formulated intramuscular administration.
59. A method for eliciting a pharmacodynamic effect in a subject in need thereof, the method comprises administering to the subject a composition comprising:
- a) a nucleic acid construct of any one of claims 1-33;
 - b) a recombinant cell of any one of claims 34-41; and/or

- d) a pharmaceutical composition of any one of claims 48-58.
60. The method of claim 59, 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.
61. The method of claim 60, wherein the pharmacodynamic effect comprises preventing viral infection and/or decreasing viral load in the subject.
62. The method of claim 60, wherein the pharmacodynamic effect comprises preventing and/or decreasing disease severity in the subject.
63. The method of any one of claims 60-62, wherein the pharmacodynamic effect comprises eliciting an immune response in the subject.
64. The method of claim 63, wherein the immune response comprises one or more of the following: an anti-EBV antibody response, a neutralizing antibody response, and an anti-EBV T cell response.
65. A method for preventing and/or treating a health condition in a subject in need thereof, the method comprises prophylactically or therapeutically administering to the subject a composition comprising:
- a) a nucleic acid construct of any one of claims 1-33;
 - b) a recombinant cell of any one of claims 34-41; and/or
 - c) a pharmaceutical composition of any one of claims 48-58.
66. The method of any one of claim 59-65, wherein the administered composition elicits an immune response in the subject.
67. The method of any one of claim 59-66, wherein the administered composition results in an increased production of interferon in the subject.
68. The method of any one of claim 59-67, wherein the subject is having or suspected of having an EBV-associated disease.

69. The method of claim 68, wherein the EBV-associated disease is infectious mononucleosis (IM), an autoimmune disease, or a cancer.
70. The method of any one of claim 59-69, wherein the composition is administered to the subject individually as a single agent prophylaxis or therapy (monotherapy) or as a first therapy in combination with at least one additional therapies.
71. The method of claim 70, 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.
72. A kit for eliciting a pharmacodynamic effect, eliciting an immune response, and/or for the prevention and/or treatment of a health condition, the kit comprising:
- a) a nucleic acid construct of any one of claims 1-33;
 - b) a recombinant cell of any one of claims 34-41; and/or
 - d) a pharmaceutical composition of any one of claims 48-58.
- and instructions for performing the method of any one of claims 59-71.
73. The kit of claim 72, wherein the health condition is an EBV-associated disease.
74. The kit of claim 73, wherein the EBV-associated disease is infectious mononucleosis (IM), an autoimmune disease, or a cancer.