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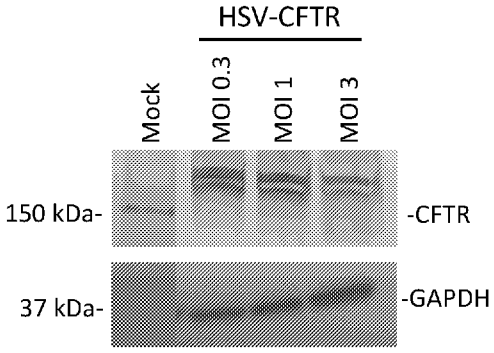
(54) Title: COMPOSITIONS AND METHODS FOR DELIVERING CFTR POLYPEPTIDES

(57) Abstract: The present disclosure provides recombinant nucleic acids comprising one or more polynucleotides encoding a cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide (e.g., a human CFTR polypeptide); viruses comprising the recombinant nucleic acids; compositions and formulations comprising the recombinant nucleic acids and/or viruses; methods of their use (e.g., for the treatment of a chronic lung disease, such as cystic fibrosis); and articles of manufacture or kits thereof.

FIG. 3



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COMPOSITIONS AND METHODS FOR DELIVERING CFTR POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial No. 62/802,871, filed February 8, 2019, which is incorporated herein by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 7613420001140SEQLIST.txt, date recorded: January 17, 2020, size: 44 KB).

FIELD OF THE INVENTION

[0003] The present disclosure relates, in part, to recombinant nucleic acids comprising one or more polynucleotides encoding a cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide, viruses comprising the same, pharmaceutical compositions and formulations thereof, and methods of their use (*e.g.,* for providing prophylactic, palliative, or therapeutic relief of one or more signs or symptoms of a chronic lung disease, such as cystic fibrosis).

BACKGROUND

[0004] Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride and bicarbonate channel that is critical for lung homeostasis. Reduction or loss of CFTR channel function often leads to mucus stasis, chronic bacterial infections, and the accompanying chronic inflammatory responses that promote progressive lung destruction. Decreases in CFTR expression have been suggested to be a component of the lung pathology observed in chronic obstructive pulmonary disease (COPD) patients, and loss-of-function mutations in the *CFTR* gene lead to the dire consequences associated with cystic fibrosis (CF). 2,000+ unique mutations in the *CFTR* gene have been described.

[0005] CF is an inherited disease characterized by the buildup of thick, sticky mucus that can damage many of the body's organs; however, the most severe pathological consequences are lung-associated. CF patients present with dehydrated mucus in the lungs that leads to airway obstruction, chronic bacterial infections (and associated inflammatory responses), bronchiectasis, and ultimately, respiratory failure. Presently, more than 70,000 people are

living with cystic fibrosis worldwide. Historically, children born with CF died as infants, and as recently as 1980 the median survival was less than 20 years. While medical advances in the past three decades have drastically improved both the quality-of-life and life expectancy of CF patients (40.6 years in the United States as of 2013), there exists a clear need for novel treatment options targeting molecular correction of CFTR deficiencies observed in CF patients, as well as in patients suffering from other chronic lung diseases like COPD.

[0006] All references cited herein, including patent applications, patent publications, non-patent literature, and NCBI/UniProtKB/Swiss-Prot accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

BRIEF SUMMARY

[0007] In order to meet these and other needs, provided herein are recombinant nucleic acids (*e.g.,* recombinant herpes virus genomes) encoding one or more CFTR polypeptides for use in viruses (*e.g.,* herpes viruses), pharmaceutical compositions and formulations, medicaments, and/or methods useful for treating CFTR deficiencies in a subject in need thereof and/or for providing prophylactic, palliative, or therapeutic relief of one or more signs or symptoms of a chronic lung disease, such as cystic fibrosis.

[0008] The present inventors have shown that the recombinant viruses described herein were capable of effectively transducing airway epithelial cells derived from a CF patient and successfully expressing their encoded exogenous human CFTR polypeptides (*see e.g.,* Example 2). In addition, the present inventors have shown that the recombinant viruses described herein expressed full-length, functional human CFTR which was appropriately trafficked to the plasma membrane (*see e.g.,* Example 2). Furthermore, the present inventors have shown that the recombinant viruses described herein rescued the diseased phenotype in clinically relevant 3D organotypic cultures prepared from biopsies harvested from multiple CF patients harboring various underlying *CFTR* mutations (*see e.g.,* Example 3). Moreover, the present inventors have shown that recombinant HSV vectors can be administered to the lungs of immunocompetent animals via multiple routes, and further, that a non-invasive inhaled route of administration expressed similar levels of an encoded transgene in, while inducing less cell invasion into, the lungs (*see e.g.,* Example 4). Without wishing to be bound by theory, it is believed that increasing, augmenting, and/or supplementing the levels of CFTR polypeptides in one or more cells (*e.g.,* one or more airway epithelial cells and/or one

or more cells of the submucosal glands) of an individual in need thereof by administering one or more of the recombinant nucleic acids, viruses, medicaments, and/or compositions described herein will: 1) reduce or prevent mucus buildup in one or more organs (*e.g.,* the lungs) of the individual; 2) reduce or prevent airway obstruction in the individual; 3) reduce or prevent chronic bacterial infections and/or the associated chronic inflammation in the lungs of the individual; 4) reduce or prevent bronchiectasis in the individual; 5) reduce, inhibit, or treat progressive lung destruction in the individual; and/or 6) provide prophylactic, palliative, or therapeutic relief of one or more signs or symptoms of a chronic lung disease (*e.g.,* cystic fibrosis, COPD, *etc.*).

[0009] Accordingly, certain aspects of the present disclosure relate to a recombinant herpes virus genome comprising one or more polynucleotides encoding a cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide. In some embodiments, the recombinant herpes virus genome is replication competent. In some embodiments, the recombinant herpes virus genome is replication defective. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within one or more viral gene loci. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes virus genome is selected from a recombinant herpes simplex virus genome, a recombinant varicella zoster virus genome, a recombinant human cytomegalovirus genome, a recombinant herpesvirus 6A genome, a recombinant herpesvirus 6B genome, a recombinant herpesvirus 7 genome, a recombinant Kaposi's sarcoma-associated herpesvirus genome, and any combinations or derivatives thereof.

[0010] In some embodiments that may be combined with any of the preceding embodiments, the CFTR polypeptide is a human CFTR polypeptide. In some embodiments that may be combined with any of the preceding embodiments, the CFTR polypeptide comprises a 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 the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments that may be combined with any of the preceding embodiments, the CFTR polypeptide comprises a 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 the amino acid sequence of SEQ ID

NO: 5. In some embodiments, the CFTR polypeptide comprises the amino acid sequence of SEQ ID NO: 5.

[0011] In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes virus genome is a recombinant herpes simplex virus genome. In some embodiments, the recombinant herpes simplex virus genome is a recombinant type 1 herpes simplex virus (HSV-1) genome, a recombinant type 2 herpes simplex virus (HSV-2) genome, or any derivatives thereof. In some embodiments, the recombinant herpes simplex virus genome is a recombinant HSV-1 genome.

[0012] In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation. In some embodiments, the inactivating mutation is in a herpes simplex virus gene. In some embodiments, the inactivating mutation is a deletion of the coding sequence of the herpes simplex virus gene. In some embodiments, the herpes simplex virus gene is selected from the Infected Cell Protein (ICP) 0 (one or both copies), ICP4 (one or both copies), ICP22, ICP27, ICP47, thymidine kinase (tk), Long Unique Region (UL) 41, and UL55. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in one or both copies of the ICP4 gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP22 gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the UL41 gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in one or both copies of the ICP0 gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP27 gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP47 gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the UL55 gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the Joint region. In some

embodiments, the recombinant herpes simplex virus genome comprises a deletion of the Joint region.

[0013] In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within one or more viral gene loci. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within one or both of the ICP4 viral gene loci. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the ICP22 viral gene locus. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the UL41 viral gene locus. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within one or both of the ICP0 viral gene loci. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the ICP27 viral gene locus. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the ICP47 viral gene locus. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the UL55 viral gene locus.

[0014] In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes virus genome has reduced cytotoxicity when introduced into a target cell as compared to a corresponding wild-type herpes virus genome. In some embodiments, the target cell is a human cell. In some embodiments that may be combined with any of the preceding embodiments, the target cell is a cell of the respiratory tract. In some embodiments that may be combined with any of the preceding embodiments, the target cell is an airway epithelial cell or a cell of the submucosal glands.

[0015] Other aspects of the present disclosure relate to a herpes virus comprising any of the recombinant herpes virus genomes described herein. In some embodiments, the herpes

virus is replication competent. In some embodiments, the herpes virus is replication defective. In some embodiments that may be combined with any of the preceding embodiments, the herpes virus has reduced cytotoxicity as compared to a corresponding wild-type herpes virus. In some embodiments, the herpes virus has reduced cytotoxicity when introduced into a target cell as compared to a corresponding wild-type herpes virus. In some embodiments, the target cell is a human cell. In some embodiments that may be combined with any of the preceding embodiments, the target cell is a cell of the respiratory tract. In some embodiments that may be combined with any of the preceding embodiments, the target cell is an airway epithelial cell or a cell of the submucosal glands. In some embodiments that may be combined with any of the preceding embodiments, the herpes virus is selected from a herpes simplex virus, a varicella zoster virus, a human cytomegalovirus, a herpesvirus 6A, a herpesvirus 6B, a herpesvirus 7, a Kaposi's sarcoma-associated herpesvirus, and any combinations or derivatives thereof. In some embodiments that may be combined with any of the preceding embodiments, the herpes virus is a herpes simplex virus. In some embodiments, the herpes simplex virus is an HSV-1, an HSV-2, or any derivatives thereof. In some embodiments, the herpes simplex virus is an HSV-1.

[0016] Other aspects of the present disclosure relate to a pharmaceutical composition comprising any of the recombinant herpes virus genomes described herein and/or any of the herpes viruses described herein and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is suitable for topical, transdermal, subcutaneous, intradermal, oral, intranasal, intratracheal, sublingual, buccal, rectal, vaginal, inhaled, intravenous, intraarterial, intramuscular, intracardiac, intraosseous, intraperitoneal, transmucosal, intravitreal, subretinal, intraarticular, peri-articular, local, and/or epicutaneous administration. In some embodiments, the pharmaceutical composition is suitable for oral, intranasal, intratracheal, and/or inhaled administration. In some embodiments, the pharmaceutical composition is suitable for inhaled administration. In some embodiments, the pharmaceutical composition is suitable for non-invasive inhaled administration. In some embodiments, the pharmaceutical composition is suitable for use in a dry powder inhaler, a pressurized metered dose inhaler, a soft mist inhaler, a nebulizer, an electrohydrodynamic aerosol device, or any combinations thereof. In some embodiments, the pharmaceutical composition is suitable for nebulization (*e.g.*, using a vibrating mesh nebulizer). In some embodiments that may be combined with any of the preceding embodiments, the pharmaceutical composition comprises a phosphate buffer. In some embodiments that may be

combined with any of the preceding embodiments, the pharmaceutical composition comprises glycerol. In some embodiments that may be combined with any of the preceding embodiments, the pharmaceutical composition comprises a lipid carrier. In some embodiments that may be combined with any of the preceding embodiments, the pharmaceutical composition comprises a nanoparticle carrier.

[0017] Other aspects of the present disclosure relate to the use of any of the recombinant nucleic acids, herpes viruses, and/or pharmaceutical compositions described herein as a medicament.

[0018] Other aspects of the present disclosure relate to the use of any of the recombinant nucleic acids, herpes viruses, and/or pharmaceutical compositions described herein in a therapy.

[0019] Other aspects of the present disclosure relate to the use of any of the recombinant nucleic acids, herpes viruses, and/or pharmaceutical composition described herein in the production or manufacture of a medicament for treating one or more signs or symptoms of a CFTR deficiency and/or a chronic lung disease (*e.g.,* cystic fibrosis, COPD, *etc.*).

[0020] Other aspects of the present disclosure relate to a method of enhancing, increasing, augmenting, and/or supplementing the levels of a CFTR polypeptide in one or more cells of a subject, the method comprising administering to the subject an effective amount of any of the recombinant herpes virus genomes described herein, any of the herpes viruses described herein, and/or any of the pharmaceutical compositions described herein. In some embodiments, the one or more cells are one or more cells of the respiratory tract. In some embodiments, the one or more cells are one or more airway epithelial cells and/or one or more cells of the submucosal glands. In some embodiments that may be combined with any of the preceding embodiments, the subject suffers from a chronic lung disease. In some embodiments, the chronic lung disease is cystic fibrosis or chronic obstructive pulmonary disease (COPD). In some embodiments that may be combined with any of the preceding embodiments, the subject is a human. In some embodiments that may be combined with any of the preceding embodiments, the subject's genome comprises a loss-of-function mutation in a *CFTR* gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered orally, intranasally, intratracheally, sublingually, buccally, topically, rectally, via inhalation, transdermally, subcutaneously, intradermally, intravenously, intraarterially, intramuscularly, intracardially, intraosseously, intraperitoneally,

transmucosally, vaginally, intravitreally, intraorbitally, subretinally, intraarticularly, peri-articularly, locally, and/or epicutaneously to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered orally, intranasally, intratracheally, or via inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via non-invasive inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered using a dry powder inhaler, a pressurized metered dose inhaler, a soft mist inhaler, a nebulizer, or an electrohydrodynamic aerosol device. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via a nebulizer (*e.g.,* a vibrating mesh nebulizer).

[0021] Other aspects of the present disclosure relate to a method of reducing or inhibiting progressive lung destruction in a subject in need thereof, the method comprising administering to the subject an effective amount of any of the recombinant herpes virus genomes described herein, any of the herpes viruses described herein, and/or any of the pharmaceutical compositions described herein. In some embodiments, the subject suffers from a chronic lung disease. In some embodiments, the chronic lung disease is cystic fibrosis or chronic obstructive pulmonary disease (COPD). In some embodiments that may be combined with any of the preceding embodiments, the subject is a human. In some embodiments that may be combined with any of the preceding embodiments, the subject's genome comprises a loss-of-function mutation in a *CFTR* gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered orally, intranasally, intratracheally, sublingually, buccally, topically, rectally, via inhalation, transdermally, subcutaneously, intradermally, intravenously, intraarterially, intramuscularly, intracardially, intraosseously, intraperitoneally, transmucosally, vaginally, intravitreally, intraorbitally, subretinally, intraarticularly, peri-articularly, locally, and/or epicutaneously to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered orally, intranasally, intratracheally, or via inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the

herpes virus, and/or the pharmaceutical composition is administered via inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via non-invasive inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered using a dry powder inhaler, a pressurized metered dose inhaler, a soft mist inhaler, a nebulizer, or an electrohydrodynamic aerosol device. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via a nebulizer (*e.g.,* a vibrating mesh nebulizer).

[0022] Other aspects of the present disclosure relate to a method of providing prophylactic, palliative, or therapeutic relief of one or more signs or symptoms of cystic fibrosis in a subject in need thereof, the method comprising administering to the subject an effective amount of any of the recombinant herpes virus genomes described herein, any of the herpes viruses described herein, and/or any of the pharmaceutical compositions described herein. In some embodiments, the one or more signs or symptoms of cystic fibrosis are selected from a persistent cough that produces thick mucus, thick sticky mucus that builds up in the airways, wheezing, breathlessness, sinusitis, repeated lung infections, inflamed nasal passages, bronchiectasis, nasal polyps, hemoptysis, pneumothorax, pancreatitis, recurring pneumonia, respiratory failure, and any combinations thereof. In some embodiments that may be combined with any of the preceding embodiments, the subject is a human. In some embodiments that may be combined with any of the preceding embodiments, the subject's genome comprises a loss-of-function mutation in a *CFTR* gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered orally, intranasally, intratracheally, sublingually, buccally, topically, rectally, via inhalation, transdermally, subcutaneously, intradermally, intravenously, intraarterially, intramuscularly, intracardially, intraosseously, intraperitoneally, transmucosally, vaginally, intravitreally, intraorbitally, subretinally, intraarticularly, peri-articularly, locally, and/or epicutaneously to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered orally, intranasally, intratracheally, or via inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or

the pharmaceutical composition is administered via non-invasive inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered using a dry powder inhaler, a pressurized metered dose inhaler, a soft mist inhaler, a nebulizer, or an electrohydrodynamic aerosol device. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via a nebulizer (*e.g.,* a vibrating mesh nebulizer).

[0023] Other aspects of the present disclosure relate to a method of providing prophylactic, palliative, or therapeutic relief of one or more signs or symptoms of COPD in a subject in need thereof, the method comprising administering to the subject an effective amount of any of the recombinant herpes virus genomes described herein, any of the herpes viruses described herein, and/or any of the pharmaceutical compositions described herein. In some embodiments, the one or more signs or symptoms of COPD are selected from shortness of breath, wheezing, chest tightness, excess mucus in the lungs, a chronic cough, cyanosis, frequent respiratory infections, and any combinations thereof. In some embodiments that may be combined with any of the preceding embodiments, the subject is a human. In some embodiments that may be combined with any of the preceding embodiments, the subject's genome comprises a loss-of-function mutation in a *CFTR* gene. In some embodiments that may be combined with any of the preceding embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered orally, intranasally, intratracheally, sublingually, buccally, topically, rectally, via inhalation, transdermally, subcutaneously, intradermally, intravenously, intraarterially, intramuscularly, intracardially, intraosseously, intraperitoneally, transmucosally, vaginally, intravitreally, intraorbitally, subretinally, intraarticularly, peri-articularly, locally, and/or epicutaneously to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered orally, intranasally, intratracheally, or via inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via non-invasive inhalation to the subject. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered using a dry powder inhaler, a pressurized metered dose inhaler, a soft mist inhaler, a nebulizer, or an electrohydrodynamic aerosol

device. In some embodiments, the recombinant herpes virus genome, the herpes virus, and/or the pharmaceutical composition is administered via a nebulizer (*e.g.,* a vibrating mesh nebulizer).

[0024] Other aspects of the present disclosure relate to an article of manufacture or kit comprising any of the recombinant herpes virus genomes, herpes viruses, medicaments, and/or pharmaceutical compositions described herein and instructions for administering the recombinant herpes virus genome, herpes virus, medicament, or pharmaceutical composition. In some embodiments, the article of manufacture or kit further comprises a device for aerosolizing the recombinant herpes virus genome, herpes virus, medicament, and/or pharmaceutical composition. In some embodiments, the device is a dry powder inhaler, a pressurized metered dose inhaler, a soft mist inhaler, a nebulizer, or an electrohydrodynamic aerosol device. In some embodiments, the device is a nebulizer (*e.g.,* a vibrating mesh nebulizer).

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **FIGS. 1A-1I** show schematics of wild-type and modified herpes simplex virus genomes. **FIG. 1A** shows a wild-type herpes simplex virus genome. **FIG. 1B** shows a modified herpes simplex virus genome comprising deletions of the coding sequence of ICP4 (both copies), with an expression cassette containing a nucleic acid encoding a human CFTR polypeptide integrated at each of the ICP4 loci. **FIG. 1C** shows a modified herpes simplex virus genome comprising deletions of the coding sequences of ICP4 (both copies) and UL41, with an expression cassette containing a nucleic acid encoding a human CFTR polypeptide integrated at each of the ICP4 loci. **FIG. 1D** shows a modified herpes simplex virus genome comprising deletions of the coding sequences of ICP4 (both copies) and UL41, with an expression cassette containing a nucleic acid encoding a CFTR polypeptide integrated at the UL41 locus. **FIG. 1E** shows a modified herpes simplex virus genome comprising deletions of the coding sequences of ICP4 (both copies) and ICP22, with an expression cassette containing a nucleic acid encoding a human CFTR polypeptide integrated at each of the ICP4 loci. **FIG. 1F** shows a modified herpes simplex virus genome comprising deletions of the coding sequences of ICP4 (both copies) and ICP22, with an expression cassette containing a nucleic acid encoding a CFTR polypeptide integrated at the ICP22 locus. **FIG. 1G** shows a modified herpes simplex virus genome comprising deletions of the coding sequences of ICP4 (both copies), UL41, and ICP22, with an expression cassette containing a nucleic acid

encoding a human CFTR polypeptide integrated at each of the ICP4 loci. **FIG. 1H** shows a modified herpes simplex virus genome comprising deletions of the coding sequences of ICP4 (both copies), UL41, and ICP22, with an expression cassette containing a nucleic acid encoding a CFTR polypeptide integrated at the UL41 locus. **FIG. 1I** shows a modified herpes simplex virus genome comprising deletions of the coding sequences of ICP4 (both copies), UL41, and ICP22, with an expression cassette containing a nucleic acid encoding a CFTR polypeptide integrated at the ICP22 locus.

[0026] **FIG. 2** shows expression of human *CFTR* in cystic fibrosis (CF) patient-derived primary small airway epithelial cells (SAECs) infected at the indicated multiplicities of infection (MOIs) with an HSV-CFTR vector, as assessed by qRT-PCR analysis. Mock infected CF SAECs were used as a negative control. Data is presented as the average of two replicates ± SEM.

[0027] **FIG. 3** shows expression of human CFTR protein in CF patient-derived primary SAECs infected at the indicated MOIs with an HSV-CFTR vector, as assessed by western blot analysis. Mock infected CF SAECs were used as a negative control. GAPDH was used as a loading control.

[0028] **FIGS. 4A-4B** show representative immunofluorescence images of human CFTR protein expression in mock infected or HSV-CFTR infected primary CF patient SAECs. **FIG. 4A** shows the dose-dependent increase in human CFTR protein expression upon infection of primary CF SAECs with increasing MOIs of HSV-CFTR. **FIG. 4B** shows the relative cellular localization of human CFTR protein in HSV-CFTR infected (MOI 3) or mock infected (MOI 0) primary CF SAECs. DAPI staining was used to visualize nuclei.

[0029] **FIG. 5** shows human CFTR protein functionality in CF patient-derived primary SAECs infected at the indicated MOIs with an HSV-CFTR vector, as assessed by a fluorescent dye uptake assay. Mock infected CF SAECs were used as a negative control. Data is presented as the average ± SEM.

[0030] **FIGS. 6A-6C** show analyses of G542X/G542X cystic fibrosis patient-derived intestinal organoids (PDOs) infected with HSV-CFTR at the indicated MOIs. Vehicle alone or an mCherry-encoding HSV vector (mCherry) were used as negative controls; G418 was used as a positive control. **FIG. 6A** shows representative brightfield images of G542X/G542X PDOs 24 hours after vehicle treatment, or after transduction with either HSV-CFTR or HSV-mCherry at an MOI of 10. Vehicle-treated PDOs isolated from a healthy individual (wild-type) were included and imaged as a comparator. **FIG. 6B** shows

representative images of calcein-stained organoids and the quantification of average organoid size prior to forskolin (Frsk) addition (t=0). **FIG. 6C** shows representative images of calcein-stained organoids and the quantification of average organoid size 60 minutes after 2μM Frsk addition (t=60). ***p<0.001; ****p<0.0001.

[0031] **FIGS. 7A-7B** show analyses of F508del/F508del cystic fibrosis patient-derived intestinal organoids (PDOs) infected with HSV-CFTR at the indicated MOIs. Vehicle alone or an mCherry-encoding HSV vector (mCherry) were used as negative controls; Orkambi® was used as a positive control. **FIG. 7A** shows representative images of calcein-stained organoids and the quantification of average organoid size prior to forskolin (Frsk) addition (t=0). **FIG. 7B** shows representative images of calcein-stained organoids and the quantification of average organoid size 60 minutes after 2μM Frsk addition (t=60). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

[0032] **FIGS. 8A-8B** show analyses of W1282X/W1282X cystic fibrosis patient-derived intestinal organoids (PDOs) infected with HSV-CFTR at the indicated MOIs. Vehicle alone or an mCherry-encoding HSV vector (mCherry) were used as negative controls. **FIG. 8A** shows representative images of calcein-stained organoids and the quantification of average organoid size prior to forskolin (Frsk) addition (t=0). **FIG. 8B** shows representative images of calcein-stained organoids and the quantification of average organoid size 60 minutes after 2μM Frsk addition (t=60). *p<0.05; ***p<0.001.

[0033] **FIGS. 9A-9B** show analyses of F508del/F508del cystic fibrosis patient-derived intestinal organoids (PDOs) infected with HSV-CFTR at the indicated MOIs. Vehicle alone or an mCherry-encoding HSV vector (mCherry) were used as negative controls; Orkambi® was used as a positive control. **FIG. 9A** shows representative images of calcein-stained organoids and the quantification of average organoid size prior to forskolin (Frsk) addition (t=0). **FIG. 9B** shows representative images of calcein-stained organoids and the quantification of average organoid size 60 minutes after 2μM Frsk addition (t=60). ****p<0.0001.

[0034] **FIGS. 10A-10C** show mCherry nucleic acid and protein analyses in lung and trachea biopsies harvested 48 hours after intranasal or intratracheal administration of an mCherry-encoding HSV vector (HSV-mCherry) or vehicle control (mock). **FIG. 10A** shows the levels of *mCherry* transcripts present in lung and trachea biopsies, as assessed by qRT-PCR analysis. Data is presented as the average of six replicates ± SEM for HSV-mCherry; data is presented as the average of four replicates ± SEM for vehicle control. **FIG. 10B** shows

representative immunofluorescence images of mCherry protein expression in lung biopsies after intranasal administration of HSV-mCherry or vehicle control. DAPI staining was used to visualize nuclei; cytokeratin staining was used to visualize epithelial cells. **FIG. 10C** shows representative immunofluorescence images of mCherry protein expression in lung biopsies after intratracheal administration of HSV-mCherry or vehicle control. DAPI staining was used to visualize nuclei; cytokeratin staining was used to visualize epithelial cells.

DETAILED DESCRIPTION

[0035] The following description sets forth exemplary methods, parameters, and the like. It should be recognized, however, that such a description is not intended as a limitation on the scope of the present disclosure but is instead provided as a description of exemplary embodiments.

I. General techniques

[0036] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al. eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999).

II. Definitions

[0037] Before describing the present disclosure in detail, it is to be understood that the present disclosure is not limited to particular compositions or biological systems, which can,

of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0038] As used herein, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0039] As used herein, the term "and/or" may include any and all combinations of one or more of the associated listed items. For example, the term "a and/or b" may refer to "a alone", "b alone", "a or b", or "a and b"; the term "a, b, and/or c" may refer to "a alone", "b alone", "c alone", "a or b", "a or c", "b or c", "a, b, or c", "a and b", "a and c", "b and c", or "a, b, and c"; *etc.*

[0040] As used herein, the term "about" refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se.*

[0041] It is understood that aspects and embodiments of the present disclosure include "comprising", "consisting", and "consisting essentially of" aspects and embodiments.

[0042] As used herein, the terms "polynucleotide", "nucleic acid sequence", "nucleic acid", and variations thereof shall be generic to polydeoxyribonucleotides (containing 2-deoxy-D-ribose), to polyribonucleotides (containing D-ribose), to any other type of polynucleotide that is an N-glycoside of a purine or pyrimidine base, and to other polymers containing non-nucleotidic backbones, provided that the polymers contain nucleobases in a configuration that allows for base pairing and base stacking, as found in DNA and RNA. Thus, these terms include known types of nucleic acid sequence modifications, for example, substitution of one or more of the naturally occurring nucleotides with an analog, and inter-nucleotide modifications.

[0043] As used herein, a nucleic acid is "operatively linked" or "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence, or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operatively linked" or "operably linked" means that the DNA or RNA sequences being linked are contiguous.

[0044] As used herein, the term "vector" refers to discrete elements that are used to introduce heterologous nucleic acids into cells for either expression or replication thereof. An

expression vector includes vectors capable of expressing nucleic acids that are operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such nucleic acids. Thus, an expression vector may refer to a DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the nucleic acids. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and those that remain episomal or those which integrate into the host cell genome.

[0045] As used herein, an "open reading frame" or "ORF" refers to a continuous stretch of nucleic acids, either DNA or RNA, that encode a protein or polypeptide. Typically, the nucleic acids comprise a translation start signal or initiation codon, such as ATG or AUG, and a termination codon.

[0046] As used herein, an "untranslated region" or "UTR" refers to untranslated nucleic acids at the 5' and/or 3' ends of an open reading frame. The inclusion of one or more UTRs in a polynucleotide may affect post-transcriptional regulation, mRNA stability, and/or translation of the polynucleotide.

[0047] As used herein, the term "transgene" refers to a polynucleotide that is capable of being transcribed into RNA and translated and/or expressed under appropriate conditions after being introduced into a cell. In some embodiments, it confers a desired property to a cell into which it was introduced, or otherwise leads to a desired therapeutic or diagnostic outcome.

[0048] As used herein, the terms "polypeptide," "protein," and "peptide" are used interchangeably and may refer to a polymer of two or more amino acids.

[0049] As used herein, a "subject", "host", or an "individual" refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, as well as animals used in research, such as mice, rats, hamsters, rabbits, and non-human primates, *etc.* In some embodiments, the mammal is human.

[0050] As used herein, the terms "pharmaceutical formulation" or "pharmaceutical composition" refer to a preparation which is in such a form as to permit the biological activity of the active ingredient(s) to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the composition or formulation would be administered. "Pharmaceutically acceptable" excipients (*e.g.,* vehicles, additives) are those

which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient(s) employed.

[0051] As used herein, an "effective amount" is at least the minimum amount required to affect a measurable improvement or prevention of one or more symptoms of a particular disorder. An "effective amount" may vary according to factors such as the disease state, age, sex, and weight of the patient. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications used to treat symptoms of the disease, delaying the progression of the disease, and/or prolonging survival. An effective amount can be administered in one or more administrations. For purposes of the present disclosure, an effective amount of a recombinant nucleic acid, virus, and/or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a recombinant nucleic acid, virus, and/or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0052] As used herein, "treatment" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease/disorder/defect progression, ameliorating or palliating the disease/disorder/defect state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with a chronic lung disease (*e.g.,* cystic fibrosis or COPD) are mitigated or eliminated.

[0053] As used herein, the term "delaying progression of" a disease/disorder/defect refers to deferring, hindering, slowing, retarding, stabilizing, and/or postponing development of the disease/disorder/defect (*e.g.,* cystic fibrosis or COPD). This delay can be of varying lengths

or time, depending on the history of the disease/disorder/defect and/or the individual being treated. As is evident to one of ordinary skill in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease.

III. Recombinant Nucleic Acids

[0054] Certain aspects of the present disclosure relate to recombinant nucleic acids (*e.g.,* isolated recombinant nucleic acids) comprising one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, ten or more, *etc.*) polynucleotides encoding a CFTR polypeptide (*e.g.,* a human CFTR polypeptide). In some embodiments, the recombinant nucleic acid comprises one polynucleotide encoding a CFTR polypeptide. In some embodiments, the recombinant nucleic acid comprises two polynucleotides encoding a CFTR polypeptide. In some embodiments, the recombinant nucleic acid comprises three polynucleotides encoding a CFTR polypeptide.

[0055] In some embodiments, the recombinant nucleic acid is a vector. In some embodiments, the recombinant nucleic acid is a viral vector. In some embodiments, the recombinant nucleic acid is a herpes viral vector. In some embodiments, the recombinant nucleic acid is a herpes simplex virus amplicon. In some embodiments, the recombinant nucleic acid is a recombinant herpes virus genome. In some embodiments, the recombinant nucleic acid is a recombinant herpes simplex virus genome. In some embodiments, the recombinant nucleic acid is a recombinant herpes simplex virus type 1 (HSV-1) genome.

Polynucleotides encoding Cystic fibrosis transmembrane conductance regulator (CFTR) polypeptides

[0056] In some embodiments, the present disclosure relates to a recombinant nucleic acid comprising one or more polynucleotides comprising the coding sequence of a *CFTR* gene (*e.g.,* a human *CFTR* gene), or any portions thereof. The sequence of any suitable *CFTR* gene (including any isoform thereof) known in the art may be encoded by a polynucleotide of the present disclosure, including, for example, a human *CFTR* gene (*see e.g.,* NCBI Gene ID: 1080; SEQ ID NO: 1 or SEQ ID NO: 3), a chimpanzee *CFTR* gene (*see e.g.,* NCBI Gene ID: 463674), a mouse *CFTR* gene (*see e.g.,* NCBI Gene ID: 12638), a rat *CFTR* gene (*see e.g.,* NCBI Gene ID: 24255), a dog *CFTR* gene (*see e.g.,* NCBI Gene ID: 492302), a rabbit *CFTR* gene (*see e.g.,* NCBI Gene ID: 100009471), a cow *CFTR* gene (*see e.g.,* NCBI Gene ID: 281067), a rhesus monkey *CFTR* gene (*see e.g.,* NCBI Gene ID: 574346), *etc.* In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least

75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of any of the *CFTR* genes described herein or known in the art (and/or the coding sequences thereof). Methods of identifying *CFTR* gene homologs/orthologs from additional species are known to one of ordinary skill in the art, including, for example, using a nucleic acid sequence alignment program such as the BLAST® blastn suite.

[0057] In some embodiments, a polynucleotide of the present disclosure comprises a codon-optimized variant of any of the *CFTR* genes described herein or known in the art. In some embodiments, a polynucleotide of the present disclosure comprises a codon-optimized variant of the coding sequence of any of the *CFTR* genes described herein or known in the art. In some embodiments, use of a codon-optimized variant of a *CFTR* gene increases stability and/or yield of heterologous expression (RNA and/or protein) of the encoded CFTR polypeptide in a target cell (*e.g.,* a target human cell such as a human airway epithelial cell), as compared to the stability and/or yield of heterologous expression of a corresponding non-codon-optimized, wild-type sequence. Any suitable method known in the art for performing codon optimization of a sequence for expression in one or more target cells (*e.g.,* one or more cells of the lung) may be used, including, for example, by the methods described by Fath *et al.* (PLoS One. 2011 Mar 3;6(3): e17596).

[0058] In some embodiments, one or more polynucleotides of the present disclosure comprise the coding sequence of a human *CFTR* gene.

[0059] In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of SEQ ID NO: 1. In some embodiments, a polynucleotide of the present disclosure comprises the sequence of SEQ ID NO: 1.

[0060] In some embodiments, a polynucleotide of the present disclosure comprises a 5' truncation, a 3' truncation, or a fragment of the sequence of SEQ ID NO: 1. In some embodiments, the 5' truncation, 3' truncation, or fragment of the sequence of SEQ ID NO: 1 is a polynucleotide that has at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, or at least 350, at least 400, at least 450, at least 500, at least 750, at least 1000, at least 1250, at least 1500, at least 1750, at

least 2000, at least 2250, at least 2500, at least 2750, at least 3000, at least 3250, at least 3500, at least 3750, at least 4000, at least 4250, but fewer than 4443 consecutive nucleotides of SEQ ID NO: 1. In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of nucleic acids 1-4440 of SEQ ID NO: 1. In some embodiments, a polynucleotide of the present disclosure comprises the sequence of nucleic acids 1-4440 of SEQ ID NO: 1.

[0061] In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of SEQ ID NO: 3. In some embodiments, a polynucleotide of the present disclosure comprises the sequence of SEQ ID NO: 3.

[0062] In some embodiments, a polynucleotide of the present disclosure comprises a 5' truncation, a 3' truncation, or a fragment of the sequence of SEQ ID NO: 3. In some embodiments, the 5' truncation, 3' truncation, or fragment of the sequence of SEQ ID NO: 3 is a polynucleotide that has at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, or at least 350, at least 400, at least 450, at least 500, at least 750, at least 1000, at least 1250, at least 1500, at least 1750, at least 2000, at least 2250, at least 2500, at least 2750, at least 3000, at least 3250, at least 3500, at least 3750, at least 4000, at least 4250, but fewer than 4260 consecutive nucleotides of SEQ ID NO: 3. In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of nucleic acids 1-4257 of SEQ ID NO: 3. In some embodiments, a polynucleotide of the present disclosure comprises the sequence of nucleic acids 1-4257 of SEQ ID NO: 3.

[0063] In some embodiments, a polynucleotide of the present disclosure comprises the coding sequence of a codon-optimized variant of a human *CFTR* gene.

[0064] In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of SEQ ID NO: 2. In some embodiments, a polynucleotide of the present disclosure comprises the sequence of SEQ ID NO: 2.

[0065] In some embodiments, a polynucleotide of the present disclosure comprises a 5' truncation, a 3' truncation, or a fragment of the sequence of SEQ ID NO: 2. In some embodiments, the 5' truncation, 3' truncation, or fragment of the sequence of SEQ ID NO: 2 is a polynucleotide that has at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, or at least 350, at least 400, at least 450, at least 500, at least 750, at least 1000, at least 1250, at least 1500, at least 1750, at least 2000, at least 2250, at least 2500, at least 2750, at least 3000, at least 3250, at least 3500, at least 3750, at least 4000, at least 4250, but fewer than 4443 consecutive nucleotides of SEQ ID NO: 2. In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of nucleic acids 1-4440 of SEQ ID NO: 2. In some embodiments, a polynucleotide of the present disclosure comprises the sequence of nucleic acids 1-4440 of SEQ ID NO: 2.

[0066] In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of SEQ ID NO: 4. In some embodiments, a polynucleotide of the present disclosure comprises the sequence of SEQ ID NO: 4.

[0067] In some embodiments, a polynucleotide of the present disclosure comprises a 5' truncation, a 3' truncation, or a fragment of the sequence of SEQ ID NO: 4. In some embodiments, the 5' truncation, 3' truncation, or fragment of the sequence of SEQ ID NO: 4 is a polynucleotide that has at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, or at least 350, at least 400, at least 450, at least 500, at least 750, at least 1000, at least 1250, at least 1500, at least 1750, at

least 2000, at least 2250, at least 2500, at least 2750, at least 3000, at least 3250, at least 3500, at least 3750, at least 4000, at least 4250, but fewer than 4260 consecutive nucleotides of SEQ ID NO: 4. In some embodiments, a polynucleotide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the sequence of nucleic acids 1-4257 of SEQ ID NO: 4. In some embodiments, a polynucleotide of the present disclosure comprises the sequence of nucleic acids 1-4257 of SEQ ID NO: 4.

[0068] In some embodiments, a polynucleotide of the present disclosure comprises a 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 a nucleic acid sequence selected from SEQ ID NOS: 1-4. In some embodiments, a polynucleotide of the present disclosure comprises a sequence selected from SEQ ID NOS: 1-4. In some embodiments, a polynucleotide of the present disclosure comprises a 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 a nucleic acid sequence selected from SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, a polynucleotide of the present disclosure comprises a sequence selected from SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, a polynucleotide of the present disclosure comprises a 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 a nucleic acid sequence selected from SEQ ID NO: 3 or SEQ ID NO: 4. In some embodiments, a polynucleotide of the present disclosure comprises a sequence selected from SEQ ID NO: 3 or SEQ ID NO: 4.

[0069] A polynucleotide of the present disclosure (*e.g.*, encoding a human CFTR polypeptide) may further encode additional coding and non-coding sequences. Examples of additional coding and non-coding sequences may include, but are not limited to, sequences encoding additional polypeptide tags (*e.g.*, encoded in-frame with the CFTR protein in order to produce a fusion protein), introns (*e.g.*, native, modified, or heterologous introns), 5' and/or 3' UTRs (*e.g.*, native, modified, or heterologous 5' and/or 3' UTRs), and the like. Examples of suitable polypeptide tags may include, but are not limited, to any combination of

purification tags, such as his-tags, flag-tags, maltose binding protein and glutathione-S-transferase tags, detection tags, such as tags that may be detected photometrically (*e.g.,* green fluorescent protein, red fluorescent protein, *etc.*) and tags that have a detectable enzymatic activity (*e.g.,* alkaline phosphatase, *etc.*), tags containing secretory sequences, signal sequences, leader sequences, and/or stabilizing sequences, protease cleavage sites (*e.g.,* furin cleavage sites, TEV cleavage sites, Thrombin cleavage sites, *etc.*), and the like. In some embodiments, the 5' and/or 3'UTRs increase the stability, localization, and/or translational efficiency of the polynucleotides. In some embodiments, the 5' and/or 3'UTRs improve the level and/or duration of protein expression. In some embodiments, the 5' and/or 3'UTRs include elements (*e.g.,* one or more miRNA binding sites, *etc.*) that may block or reduce off-target expression (*e.g.,* inhibiting expression in specific cell types (*e.g.,* neuronal cells), at specific times in the cell cycle, at specific developmental stages, *etc.*). In some embodiments, the 5' and/or 3'UTRs include elements (*e.g.,* one or more miRNA binding sites, *etc.*) that may enhance CFTR expression in specific cell types.

[0070] In some embodiments, a polynucleotide of the present disclosure (*e.g.,* encoding a human CFTR polypeptide) is operably linked to one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, ten or more, *etc.*) regulatory sequences. The term "regulatory sequence" may include enhancers, insulators, promoters, and other expression control elements (*e.g.,* polyadenylation signals). Any suitable enhancer(s) known in the art may be used, including, for example, enhancer sequences from mammalian genes (such as globin, elastase, albumin, α-fetoprotein, insulin and the like), enhancer sequences from a eukaryotic cell virus (such as SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, adenovirus enhancers, and the like), and any combinations thereof. Any suitable insulator(s) known in the art may be used, including, for example, herpes simplex virus (HSV) chromatin boundary (CTRL/CTCF-binding/insulator) elements CTRL1 and/or CTRL2, chicken hypersensitive site 4 insulator (cHS4), human HNRPA2B1—CBX3 ubiquitous chromatin opening element (UCOE), the scaffold/matrix attachment region (S/MAR) from the human interferon beta gene (IFNB1), and any combinations thereof. Any suitable promoter (*e.g.,* suitable for transcription in mammalian host cells) known in the art may be used, including, for example, promoters obtained from the genomes of viruses (such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus, Simian Virus 40

(SV40), and the like), promoters from heterologous mammalian genes (such as the actin promoter (*e.g.,* the β-actin promoter), a ubiquitin promoter (*e.g.,* a ubiquitin C (UbC) promoter), a phosphoglycerate kinase (PGK) promoter, an immunoglobulin promoter, from heat-shock protein promoters, and the like), promoters from native and/or homologous mammalian genes (*e.g.,* a human *CFTR* gene promoter), synthetic promoters (such as the CAGG promoter), and any combinations thereof, provided such promoters are compatible with the host cells. Regulatory sequences may include those which direct constitutive expression of a nucleic acid, as well as tissue-specific regulatory and/or inducible or repressible sequences.

[0071] In some embodiments, a polynucleotide of the present disclosure is operably linked to one or more heterologous promoters. In some embodiments, the one or more heterologous promoters are one or more of constitutive promoters, tissue-specific promoters, temporal promoters, spatial promoters, inducible promoters and repressible promoters. In some embodiments, the one or more heterologous promoters are one or more of the human cytomegalovirus (HCMV) immediate early promoter, the human elongation factor-1 (EF1) promoter, the human β-actin promoter, the human UbC promoter, the human PGK promoter, the synthetic CAGG promoter, and any combinations thereof. In some embodiments, a polynucleotide of the present disclosure (*e.g.,* encoding a human CFTR polypeptide) is operably linked to an HCMV promoter.

[0072] In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a Collagen alpha-1 (VII) chain polypeptide (COL7). In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a Lysyl hydroxylase 3 polypeptide (LH3). In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a Keratin type I cytoskeletal 17 polypeptide (KRT17). In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a transglutaminase (TGM) polypeptide (*e.g.,* a human transglutaminase polypeptide such as a human TGM1 polypeptide and/or a human TGM5 polypeptide). In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a cosmetic protein (*e.g.,* collagen proteins, fibronectins, elastins, lumicans, vitronectins/vitronectin receptors, laminins, neuromodulators, fibrillins, additional dermal extracellular matrix proteins, *etc.*). In some embodiments, a polynucleotide of the present disclosure does not

comprise the coding sequence of (*e.g.,* a transgene encoding) an antibody (*e.g.,* a full-length antibody, an antibody fragment, *etc.*). In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a Serine Protease Inhibitor Kazal-type (SPINK) polypeptide (*e.g.,* a human SPINK polypeptide, such as a SPINK5 polypeptide). In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a filaggrin or filaggrin 2 polypeptide (*e.g.,* a human filaggrin or filaggrin 2 polypeptide). In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a Collagen alpha-1 (VII) chain polypeptide, a Lysyl hydroxylase 3 polypeptide, a Keratin type I cytoskeletal 17 polypeptide, and/or any chimeric polypeptides thereof. In some embodiments, a polynucleotide of the present disclosure does not comprise the coding sequence of (*e.g.,* a transgene encoding) a Collagen alpha-1 (VII) chain polypeptide, a Lysyl hydroxylase 3 polypeptide, a Keratin type I cytoskeletal 17 polypeptide, a transglutaminase (TGM) polypeptide, a filaggrin polypeptide, a cosmetic protein, an antibody, a SPINK polypeptide, and/or any chimeric polypeptides thereof.

Cystic fibrosis transmembrane conductance regulator (CFTR) polypeptides

[0073] In some embodiments, the present disclosure relates to one or more polynucleotides encoding a CFTR polypeptide (*e.g.,* a human CFTR polypeptide), or any portions thereof. Any suitable CFTR polypeptide known in the art may be encoded by a polynucleotide of the present disclosure, including, for example, a human CFTR polypeptide (*see e.g.,* UniProt accession number P13569; SEQ ID NO: 5 or SEQ ID NO: 6), a chimpanzee CFTR polypeptide (*see e.g.,* UniProt accession number Q2QLE5), a mouse CFTR polypeptide (*see e.g.,* UniProt accession number P26361), a rat CFTR polypeptide (*see e.g.,* UniProt accession number P34158), a rabbit CFTR polypeptide (*see e.g.,* UniProt accession number Q00554), a rhesus monkey CFTR polypeptide (*see e.g.,* UniProt accession number Q00553), *etc.* In some embodiments, a CFTR polypeptide of the present disclosure comprises a sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 the amino acid sequence of any of the CFTR polypeptides described herein or known in the art. Methods of identifying CFTR polypeptide homologs/orthologs from

additional species are known to one of ordinary skill in the art, including, for example, using an amino acid sequence alignment program such as the BLAST® blastp suite or OrthoDB.

[0074] In some embodiments, a CFTR polypeptide of the present disclosure is a human CFTR polypeptide.

[0075] In some embodiments, a polynucleotide encoding a human CFTR polypeptide is a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% identity to the sequence of SEQ ID NO: 5. In some embodiments, a polynucleotide encoding a human CFTR polypeptide is a polynucleotide that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 5.

[0076] In some embodiments, a polynucleotide encoding a human CFTR polypeptide is a polynucleotide that encodes an N-terminal truncation, a C-terminal truncation, or a fragment of the amino acid sequence of SEQ ID NO: 5. N-terminal truncations, C-terminal truncations, or fragments may comprise at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 30, at least 40, at least 50, at least 75, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, but fewer than 1480, consecutive amino acids of SEQ ID NO: 5.

[0077] In some embodiments, a polynucleotide encoding a human CFTR polypeptide is a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% identity to the sequence of SEQ ID NO: 6. In some embodiments, a polynucleotide encoding a human CFTR polypeptide is a polynucleotide that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6.

[0078] In some embodiments, a polynucleotide encoding a human CFTR polypeptide is a polynucleotide that encodes an N-terminal truncation, a C-terminal truncation, or a fragment of the amino acid sequence of SEQ ID NO: 6. N-terminal truncations, C-terminal truncations, or fragments may comprise at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 30, at least 40, at least 50, at least 75, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least

600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, but fewer than 1419, consecutive amino acids of SEQ ID NO: 6.

[0079] In some embodiments, a polynucleotide of the present disclosure encoding a CFTR polypeptide is a polynucleotide that encodes a polypeptide comprising a 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 the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, a polynucleotide of the present disclosure encoding a CFTR polypeptide is a polynucleotide that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

[0080] In some embodiments, a polynucleotide of the present disclosure encoding a CFTR polypeptide (*e.g.,* a human CFTR polypeptide) expresses the CFTR polypeptide when the polynucleotide is delivered into one or more target cells of a subject (*e.g.,* one or more cells of the airway and/or lungs of the subject). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human CFTR polypeptide) enhances, increases, augments, and/or supplements the levels, function, and/or activity of a CFTR polypeptide in one or more target cells of a subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human CFTR polypeptide) reduces mucus secretion by one or more cells and/or in one or more organs (*e.g.,* the lungs) of the subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human CFTR polypeptide) reduces and/or inhibits mucus buildup in one or more organs (*e.g.,* the lungs) of the subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human CFTR polypeptide) reduces, prevents, or treats airway obstruction in a subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human CFTR polypeptide) reduces, prevents, or treats chronic bacterial infections and/or the associated chronic inflammation in the lungs of a subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human CFTR polypeptide) reduces, inhibits, prevents, or treats bronchiectasis in a subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human

CFTR polypeptide) reduces, inhibits, prevents, or treats progressive lung destruction in a subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human CFTR polypeptide) provides prophylactic, palliative, or therapeutic relief of a chronic lung disease (*e.g.,* cystic fibrosis, chronic obstructive pulmonary disorder) in a subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide). In some embodiments, expression of the CFTR polypeptide (*e.g.,* a human CFTR polypeptide) provides prophylactic, palliative, or therapeutic relief of one or more signs or symptoms of cystic fibrosis in a subject (*e.g.,* as compared to prior to expression of the CFTR polypeptide).

Recombinant nucleic acids

[0081] In some embodiments, the present disclosure relates to recombinant nucleic acids comprising any one or more of the polynucleotides described herein. In some embodiments, the recombinant nucleic acid is a vector (*e.g.,* an expression vector, a display vector, *etc.*). In some embodiments, the vector is a DNA vector or an RNA vector. Generally, vectors suitable to maintain, propagate, and/or express polynucleotides to produce one or more polypeptides in a subject may be used. Examples of suitable vectors may include, for example, plasmids, cosmids, episomes, transposons, and viral vectors (*e.g.,* adenoviral vectors, adeno-associated viral vectors, vaccinia viral vectors, Sindbis-viral vectors, measles vectors, herpes viral vectors, lentiviral vectors, retroviral vectors, *etc.*). In some embodiments, the vector is a herpes viral vector. In some embodiments, the vector is capable of autonomous replication in a host cell. In some embodiments, the vector is incapable of autonomous replication in a host cell. In some embodiments, the vector can integrate into a host DNA. In some embodiments, the vector cannot integrate into a host DNA (*e.g.,* is episomal). Methods of making vectors containing one or more polynucleotides of interest are well known to one of ordinary skill in the art, including, for example, by chemical synthesis or by artificial manipulation of isolated segments of nucleic acids (*e.g.,* by genetic engineering techniques).

[0082] In some embodiments, a recombinant nucleic acid of the present disclosure is a herpes simplex virus (HSV) amplicon. Herpes virus amplicons, including the structural features and methods of making the same, are generally known to one of ordinary skill in the art (*see e.g.,* de Silva S. and Bowers W. "Herpes Virus Amplicon Vectors". *Viruses* 2009, 1, 594-629). In some embodiments, the herpes simplex virus amplicon is an HSV-1 amplicon. In some embodiments, the herpes simplex virus amplicon is an HSV-1 hybrid amplicon.

Examples of HSV-1 hybrid amplicons may include, but are not limited to, HSV/AAV hybrid amplicons, HSV/EBV hybrid amplicons, HSV/EBV/RV hybrid amplicons, and/or HSV/*Sleeping Beauty* hybrid amplicons. In some embodiments, the amplicon is an HSV/AAV hybrid amplicon. In some embodiments, the amplicon is an HSV/*Sleeping Beauty* hybrid amplicon.

[0083] In some embodiments, a recombinant nucleic acid of the present disclosure is a recombinant herpes virus genome. The recombinant herpes virus genome may be a recombinant genome from any member of the Herpesviridae family of DNA viruses known in the art, including, for example, a recombinant herpes simplex virus genome, a recombinant varicella zoster virus genome, a recombinant human cytomegalovirus genome, a recombinant herpesvirus 6A genome, a recombinant herpesvirus 6B genome, a recombinant herpesvirus 7 genome, a recombinant Kaposi's sarcoma-associated herpesvirus genome, and any combinations or derivatives thereof. As used herein, an "inactivating mutation" may refer to any mutation that results in a gene or regulon product (RNA or protein) having reduced, undetectable, or eliminated quantity and/or function (*e.g.,* as compared to a corresponding sequence lacking the inactivating mutation). Examples of inactivating mutations may include, but are not limited to, deletions, insertions, point mutations, and rearrangements in transcriptional control sequences (promoters, enhancers, insulators, *etc.*) and/or coding sequences of a given gene or regulon. Any suitable method of measuring the quantity of a gene or regulon product known in the art may be used, including, for example, qPCR, Northern blots, RNAseq, western blots, ELISAs, *etc.* In some embodiments, the recombinant herpes virus genome comprises one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, *etc.*) inactivating mutations. In some embodiments, the one or more inactivating mutations are in one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, *etc.*) herpes virus genes. In some embodiments, the recombinant herpes virus genome is attenuated (*e.g.,* as compared to a corresponding wild-type herpes virus genome). In some embodiments, the recombinant herpes virus genome is replication competent. In some embodiments, the recombinant herpes virus genome is replication defective.

[0084] In some embodiments, the recombinant nucleic acid is a recombinant herpes simplex virus (HSV) genome. In some embodiments, the recombinant herpes simplex virus genome is a recombinant herpes simplex virus type 1 (HSV-1) genome, a recombinant herpes

simplex virus type 2 (HSV-2) genome, or any derivatives thereof. In some embodiments, the recombinant herpes simplex virus genome comprises one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, *etc.*) inactivating mutations. In some embodiments, the one or more inactivating mutations are in one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, *etc.*) herpes simplex virus genes. In some embodiments, the recombinant herpes simplex virus genome is attenuated (*e.g.,* as compared to a corresponding, wild-type herpes simplex virus genome). In some embodiments, the recombinant herpes simplex virus genome is replication competent. In some embodiments, the recombinant herpes simplex virus genome is replication defective.

[0085] In some embodiments, the recombinant herpes simplex virus genome is a recombinant HSV-1 genome. In some embodiments, the recombinant HSV-1 genome may be from any HSV-1 strain known in the art, including, for example, strains 17, Ty25, R62, S25, Ku86, S23, R11, Ty148, Ku47, H166syn, 1319-2005, F-13, M-12, 90237, F-17, KOS, 3083-2008, F12g, L2, CD38, H193, M-15, India 2011, 0116209, F-11I, 66-207, 2762, 369-2007, 3355, MacIntyre, McKrae, 7862, 7-hse, HF10, 1394,2005, 270-2007, OD4, SC16, M-19, 4J1037, 5J1060, J1060, KOS79, 132-1988, 160-1982, H166, 2158-2007, RE, 78326, F18g, F11, 172-2010, H129, F, E4, CJ994, F14g, E03, E22, E10, E06, E11, E25, E23, E35, E15, E07, E12, E14, E08, E19, E13, ATCC 2011, *etc.* (*see e.g.,* Bowen *et al.* J Virol. 2019 Apr 3;93(8)). In some embodiments, the recombinant HSV-1 genome is from the KOS strain. In some embodiments, the recombinant HSV-1 genome is not from the McKrae strain. In some embodiments, the recombinant HSV-1 genome is attenuated. In some embodiments, the recombinant HSV-1 genome is replication competent. In some embodiments, the recombinant HSV-1 genome is replication defective. In some embodiments, the recombinant HSV-1 genome comprises one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, *etc.*) inactivating mutations. In some embodiments, the one or more inactivating mutations are in one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, *etc.*) HSV-1 genes.

[0086] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in at least one, at least two, at least three, at least four, at least five,

at least six, at least seven, or all eight of the Infected Cell Protein (ICP) 0 (one or both copies), ICP4 (one or both copies), ICP22, ICP27, ICP47, thymidine kinase (tk), Long Unique Region (UL) 41 and/or UL55 herpes simplex virus genes. In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in (*e.g.,* is capable of expressing) the ICP0 (one or both copies) herpes simplex virus gene. In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in (*e.g.,* is capable of expressing) in the ICP27 herpes simplex virus gene. In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in (*e.g.,* is capable of expressing) the ICP47 herpes simplex virus gene. In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in (*e.g.,* is capable of expressing) the ICP0 (one or both copies), ICP27, and/or ICP47 herpes simplex virus genes. In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in the Joint region. In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in the ICP34.5 (one or both copies) and/or ICP47 herpes simplex virus genes (*e.g.,* to avoid production of an immune-stimulating virus). In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in the ICP34.5 herpes simplex virus gene (one or both copies). In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in the ICP47 herpes simplex virus gene. In some embodiments, the recombinant herpes simplex virus genome does not comprise an inactivating mutation in the ICP34.5 (one or both copies) and ICP47 herpes simplex virus genes. In some embodiments, the recombinant herpes simplex virus genome is not oncolytic.

[0087] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 gene (one or both copies). In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 gene (one or both copies), and further comprises an inactivating mutation in the ICP4 (one or both copies), ICP22, ICP27, ICP47, UL41, and/or UL55 genes. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 gene (one or both copies), and an inactivating mutation in the ICP4 gene (one or both copies). In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 gene (one or both copies), and an inactivating mutation in the ICP22 gene. In some embodiments, the recombinant herpes simplex virus genome

comprises an inactivating mutation in the ICP0 gene (one or both copies), and an inactivating mutation in the UL41 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 gene (one or both copies), an inactivating mutation in the ICP4 gene (one or both copies), and an inactivating mutation in the ICP22 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 gene (one or both copies), an inactivating mutation in the ICP4 gene (one or both copies), and an inactivating mutation in the UL41 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 gene (one or both copies), an inactivating mutation in the ICP22 gene, and an inactivating mutation in the UL41 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 gene (one or both copies), an inactivating mutation in the ICP4 gene (one or both copies), an inactivating mutation in the ICP22 gene, and an inactivating mutation in the UL41 gene. In some embodiments, the inactivating mutation is a deletion of the coding sequence of the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, and/or UL41 genes. In some embodiments, the recombinant herpes simplex virus genome further comprises an inactivating mutation in the ICP27, ICP47, and/or UL55 genes.

[0088] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP4 gene (one or both copies). In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP4 gene (one or both copies), and further comprises an inactivating mutation in the ICP0 (one or both copies), ICP22, ICP27, ICP47, UL41, and/or UL55 genes. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP4 gene (one or both copies), and an inactivating mutation in the ICP22 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP4 gene (one or both copies), and an inactivating mutation in the UL41 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP4 gene (one or both copies), an inactivating mutation in the ICP22 gene, and an inactivating mutation in the UL41 gene. In some embodiments, the inactivating mutation is a deletion of the coding sequence of the ICP4 (one or both copies), ICP22, and/or UL41 genes. In some embodiments, the recombinant herpes simplex virus genome further comprises an inactivating mutation in the ICP0 (one or both copies), ICP27, ICP47, and/or UL55 genes.

[0089] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP22 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP22 gene, and further comprises an inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), ICP27, ICP47, UL41, and/or UL55 genes. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP22 gene, and an inactivating mutation UL41 gene. In some embodiments, the inactivating mutation is a deletion of the coding sequence of the ICP22 and/or UL41 genes. In some embodiments, the recombinant herpes simplex virus genome further comprises an inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), ICP27, ICP47, and/or UL55 genes.

[0090] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP27 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP27 gene, and further comprises an inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, ICP47, UL41, and/or UL55 genes. In some embodiments, the inactivating mutation is a deletion of the coding sequence of the ICP27 gene.

[0091] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP47 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP47 gene, and further comprises an inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, ICP27, UL41, and/or UL55 genes. In some embodiments, the inactivating mutation is a deletion of the coding sequence of the ICP47 gene.

[0092] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the UL41 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the UL41 gene, and further comprises an inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, ICP27, ICP47, and/or UL55 genes. In some embodiments, the inactivating mutation is a deletion of the coding sequence of the UL41 gene.

[0093] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the UL55 gene. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the UL55 gene, and further comprises an inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both

copies), ICP22, ICP27, ICP47, and/or UL41 genes. In some embodiments, the inactivating mutation is a deletion of the coding sequence of the UL55 gene.

[0094] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in (*e.g.,* a deletion of) the internal repeat (Joint) region comprising the internal repeat long (IR_L) and internal repeat short (IR_S) regions. In some embodiments, inactivation (*e.g.,* deletion) of the Joint region eliminates one copy each of the ICP4 and ICP0 genes. In some embodiments, inactivation (*e.g.,* deletion) of the Joint region further inactivates (*e.g.,* deletes) the promoter for the ICP22 and ICP47 genes. If desired, expression of one or both of these genes can be restored by insertion of an immediate early promoter into the recombinant herpes simplex virus genome (*see e.g.,* Hill *et al.* (1995). Nature 375(6530): 411-415; Goldsmith *et al.* (1998). J Exp Med 187(3): 341-348). Without wishing to be bound by theory, it is believed that inactivating (*e.g.,* deleting) the Joint region may contribute to the stability of the recombinant herpes simplex virus genome and/or allow for the recombinant herpes simplex virus genome to accommodate more and/or larger transgenes.

[0095] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP4 (one or both copies), ICP22, and ICP27 genes. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP4 (one or both copies), ICP27, and UL55 genes. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP4 (one or both copies), ICP22, ICP27, ICP47, and UL55 genes. In some embodiments, the inactivating mutation in the ICP4 (one or both copies), ICP27, and/or UL55 genes is a deletion of the coding sequence of the ICP4 (one or both copies), ICP27, and/or UL55 genes. In some embodiments, the inactivating mutation in the ICP22 and ICP47 genes is a deletion in the promoter region of the ICP22 and ICP47 genes (*e.g.,* the ICP22 and ICP47 coding sequences are intact but are not transcriptionally active). In some embodiments, the recombinant herpes simplex virus genome comprises a deletion in the coding sequence of the ICP4 (one or both copies), ICP27, and UL55 genes, and a deletion in the promoter region of the ICP22 and ICP47 genes. In some embodiments, the recombinant herpes simplex virus genome further comprises an inactivating mutation in the ICP0 (one or both copies) and/or UL41 genes.

[0096] In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 (one or both copies) and ICP4 (one or both copies) genes. In some embodiments, the recombinant herpes simplex virus genome comprises an

inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), and ICP22 genes. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, and ICP27 genes. In some embodiments, the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, ICP27 and UL55 genes. In some embodiments, the inactivating mutation in the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, ICP27 and/or UL55 genes comprises a deletion of the coding sequence of the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, ICP27 and/or UL55 genes. In some embodiments, the recombinant herpes simplex virus genome further comprises an inactivating mutation in the ICP47 and/or the UL41 genes.

[0097] In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within one, two, three, four, five, six, seven or more viral gene loci. Examples of suitable viral loci may include, without limitation, the ICP0 (one or both copies), ICP4 (one or both copies), ICP22, ICP27, ICP47, tk, UL41 and/or UL55 herpes simplex viral gene loci. In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within one or both of the viral ICP4 gene loci (*e.g.,* a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in one or both of the ICP4 loci). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within the viral ICP22 gene locus (*e.g.,* a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in the ICP22 locus). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within the viral UL41 gene locus (*e.g.,* a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in the UL41 locus). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within one or both of the viral ICP0 gene loc1 (*e.g.,* a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in one or both of the ICP0 loci). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within the viral ICP27 gene locus (*e.g.,* a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in the ICP27 locus). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within

the viral ICP47 gene locus (*e.g.*, a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in the ICP47 locus). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within one or both of the viral ICP4 gene loci, and one or more polynucleotides of the present disclosure within the viral ICP22 gene locus (*e.g.*, a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in one or both of the ICP4 loci, and a polynucleotide encoding a human CFTR polypeptide in the ICP22 locus). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within one or both of the viral ICP4 gene loci, and one or more polynucleotides of the present disclosure within the viral UL41 gene locus (*e.g.*, a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in one or both of the ICP4 loci, and a polynucleotide encoding a human CFTR polypeptide in the UL41 locus). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within the viral ICP22 gene locus, and one or more polynucleotides of the present disclosure within the viral UL41 gene locus (*e.g.*, a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in the ICP22 locus, and a polynucleotide encoding a human CFTR polypeptide in the UL41 locus). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within one or both of the viral ICP4 gene loci, one or more polynucleotides of the present disclosure within the viral ICP22 gene locus, and one or more polynucleotides of the present disclosure within the viral UL41 gene locus (*e.g.*, a recombinant virus carrying a polynucleotide encoding a human CFTR polypeptide in one or both of the ICP4 loci, a polynucleotide encoding a human CFTR polypeptide in the ICP22 locus, and a polynucleotide encoding a human CFTR polypeptide in the UL41 locus). In some embodiments, a recombinant herpes simplex virus genome comprises one or more polynucleotides of the present disclosure within one or both of the viral ICP4 gene loci, one or more polynucleotides of the present disclosure within the viral ICP22 gene locus, one or more polynucleotides of the present disclosure within the viral UL41 gene locus, one or more polynucleotides of the present disclosure within one or both of the viral ICP0 gene loci, one or more polynucleotides of the present disclosure within the viral ICP27 gene locus, and/or one or more polynucleotides of the present disclosure within the viral ICP47 gene locus.

[0098] In some embodiments, the recombinant herpes virus genome (*e.g.*, a recombinant herpes simplex virus genome) has been engineered to decrease or eliminate expression of one

or more herpes virus genes (*e.g.,* one or more toxic herpes virus genes), such as one or both copies of the HSV ICP0 gene, one or both copies of the HSV ICP4 gene, the HSV ICP22 gene, the HSV UL41 gene, the HSV ICP27 gene, the HSV ICP47 gene, *etc*. In some embodiments, the recombinant herpes virus genome (*e.g.,* a recombinant herpes simplex virus genome) has been engineered to reduce cytotoxicity of the recombinant genome (*e.g.,* when introduced into a target cell) as compared to a corresponding wild-type herpes virus genome. In some embodiments, the target cell is a human cell (primary cells or a cell line derived therefrom). In some embodiments, the target cell is a cell of the mucosa. In some embodiments, the target cell is a cell of the respiratory tract (primary cells or a cell line derived therefrom). In some embodiments, the target cell is an airway epithelial cell (primary cells or a cell line derived therefrom). In some embodiments, the target cell is a cell of the lung (primary cells or a cell line derived therefrom). In some embodiments, cytotoxicity of the recombinant herpes virus genome is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% as compared to a corresponding wild-type herpes virus genome (*e.g.,* measuring the relative cytotoxicity of a recombinant ΔICP4 (one or both copies) herpes simplex virus genome vs. a wild-type herpes simplex virus genome in a target cell; measuring the relative cytotoxicity of a recombinant ΔICP4 (one or both copies)/ΔICP22 herpes simplex virus genome vs. a wild-type herpes simplex virus genome in a target cell, *etc.*). In some embodiments, cytotoxicity of the recombinant herpes virus genome is reduced by at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 250-fold, at least about 500-fold, at least about 750-fold, at least about 1000-fold, or more as compared to a corresponding wild-type herpes virus genome (*e.g.,* measuring the relative cytotoxicity of a recombinant ΔICP4 (one or both copies) herpes simplex virus genome vs. a wild-type herpes simplex virus genome in a target cell; measuring the relative cytotoxicity of a recombinant ΔICP4 (one or both copies)/ΔICP22 herpes simplex virus genome vs. a wild-type herpes simplex virus genome in a target cell, *etc.*). Methods of measuring cytotoxicity are known to one of ordinary skill in

the art, including, for example, through the use of vital dyes (formazan dyes), protease biomarkers, an MTT assay (or an assay using related tetrazolium salts such as XTT, MTS, water-soluble tetrazolium salts, *etc.*), measuring ATP content, *etc.*

[0099] In some embodiments, the recombinant herpes virus genome (*e.g.,* a recombinant herpes simplex virus genome) has been engineered to reduce its impact on target cell proliferation after exposure of a target cell to the recombinant genome, as compared to a corresponding wild-type herpes virus genome. In some embodiments, the target cell is a human cell (primary cells or a cell line derived therefrom). In some embodiments, the target cell is a cell of the mucosa. In some embodiments, the target cell is a cell of the respiratory tract (primary cells or a cell line derived therefrom). In some embodiments, the target cell is an airway epithelial cell (primary cells or a cell line derived therefrom). In some embodiments, the target cell is a cell of the lung (primary cells or a cell line derived therefrom). In some embodiments, target cell proliferation after exposure to the recombinant genome is at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% faster as compared to target cell proliferation after exposure to a corresponding wild-type herpes virus genome (*e.g.,* measuring the relative cellular proliferation after exposure to a recombinant ΔICP4 (one or both copies) herpes simplex virus genome vs. cellular proliferation after exposure to a wild-type herpes simplex virus genome in a target cell; measuring the relative cellular proliferation after exposure to a recombinant ΔICP4 (one or both copies)/ΔICP22 herpes simplex virus genome vs. a wild-type herpes simplex virus genome in a target cell, *etc.*). In some embodiments, target cell proliferation after exposure to the recombinant genome is at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 250-fold, at least about 500-fold, at least about 750-fold, or at least about 1000-fold faster as compared to target cell proliferation after exposure to a corresponding wild-type herpes virus genome (*e.g.,* measuring the relative cellular proliferation after exposure to a recombinant ΔICP4 (one or both copies) herpes simplex virus genome vs. cellular proliferation after exposure to a wild-type herpes simplex

virus genome in a target cell; measuring the relative cellular proliferation after exposure to a recombinant ΔICP4 (one or both copies)/ΔICP22 herpes simplex virus genome vs. a wild-type herpes simplex virus genome in a target cell, *etc.*). Methods of measuring cellular proliferation are known to one of ordinary skill in the art, including, for example, through the use of a Ki67 cell proliferation assay, a BrdU cell proliferation assay, *etc.*

[0100] A vector (*e.g.,* herpes viral vector) may include one or more polynucleotides of the present disclosure in a form suitable for expression of the polynucleotide in a host cell. Vectors may include one or more regulatory sequences operatively linked to the polynucleotide to be expressed (*e.g.,* as described above).

[0101] In some embodiments, a recombinant nucleic acid (*e.g.,* a recombinant herpes simplex virus genome) of the present disclosure comprises one or more of the polynucleotides described herein inserted in any orientation in the recombinant nucleic acid. If the recombinant nucleic acid comprises two or more polynucleotides described herein (*e.g.,* two or more, three or more, *etc.*), the polynucleotides may be inserted in the same orientation or opposite orientations to one another. Without wishing to be bound be theory, incorporating two polynucleotides (*e.g.,* two transgenes) into a recombinant nucleic acid (*e.g.,* a vector) in an antisense orientation may help to avoid read-through and ensure proper expression of each polynucleotide.

IV. Viruses

[0102] Certain aspects of the present disclosure relate to viruses comprising any of the polynucleotides and/or recombinant nucleic acids described herein. In some embodiments, the virus is capable of infecting one or more target cells of a subject (*e.g.,* a human). In some embodiments, the virus is suitable for delivering the polynucleotides and/or recombinant nucleic acids into one or more target cells of a subject (*e.g.,* a human). In some embodiments, the one or more target cells are human cells. In some embodiments, the one or more target cells are one or more cells with a CFTR deficiency (*e.g.,* one or more cells comprising a genomic mutation in native *CFTR* gene). In some embodiments, the one or more target cells are one or more cells of the mucosa. In some embodiments, the one or more target cells are one or more airway epithelial cells. In some embodiments, the one or more target cells are one or more cells of the respiratory tract (*e.g.,* airway epithelial cells (such as goblet cells, ciliated cells, Clara cells, neuroendocrine cells, basal cells, intermediate or parabasal cells, Serous cells, brush cells, oncocytes, non-ciliated columnar cells, and/or metaplastic cells);

alveolar cells (such as type 1 pneumocytes, type 2 pneumocytes, and/or cuboidal non-ciliated cells); salivary gland cells in bronchi (such as Serous cells, mucous cells, and/or ductal cells); *etc.*). In some embodiments, the one or more target cells are one or more cells of the lung.

[0103] Any suitable virus known in the art may be used, including, for example, adenovirus, adeno-associated virus, retrovirus, lentivirus, sendai virus, herpes virus, vaccinia virus, and/or any hybrid or derivative viruses thereof. In some embodiments, the virus is attenuated. In some embodiments, the virus is replication competent. In some embodiments, the virus is replication defective. In some embodiments, the virus has been modified to alter its tissue tropism relative to the tissue tropism of a corresponding unmodified, wild-type virus. In some embodiments, the virus has reduced cytotoxicity (*e.g.,* in a target cell) as compared to a corresponding wild-type virus. Methods of producing a virus comprising recombinant nucleic acids are well known to one of ordinary skill in the art.

[0104] In some embodiments, the virus is a member of the Herpesviridae family of DNA viruses, including, for example, a herpes simplex virus, a varicella zoster virus, a human cytomegalovirus, a herpesvirus 6A, a herpesvirus 6B, a herpesvirus 7, and a Kaposi's sarcoma-associated herpesvirus, *etc*. In some embodiments, the herpes virus is attenuated. In some embodiments, the herpes virus is replication defective. In some embodiments, the herpes virus is replication competent. In some embodiments, the herpes virus has been engineered to reduce or eliminate expression of one or more herpes virus genes (*e.g.,* one or more toxic herpes virus genes). In some embodiments, the herpes virus has reduced cytotoxicity as compared to a corresponding wild-type herpes virus. In some embodiments, the herpes virus is not oncolytic.

[0105] In some embodiments, the herpes virus is a herpes simplex virus. Herpes simplex viruses comprising recombinant nucleic acids may be produced by a process disclosed, for example, in WO2015/009952, WO2017/176336, WO2019/200163, WO2019/210219, and/or WO2020/006486. In some embodiments, the herpes simplex virus is attenuated . In some embodiments, the herpes simplex virus is replication defective. In some embodiments, the herpes simplex virus is replication competent. In some embodiments, the herpes simplex virus has been engineered to reduce or eliminate expression of one or more herpes simplex virus genes (*e.g.,* one or more toxic herpes simplex virus genes). In some embodiments, the herpes simplex virus has reduced cytotoxicity as compared to a corresponding wild-type herpes simplex virus. In some embodiments, the herpes simplex virus is not oncolytic. In some embodiments, the herpes simplex virus is an HSV-1 virus, an HSV-2, or any

derivatives thereof. In some embodiments, the herpes simplex virus is an HSV-1 virus. In some embodiments, the herpes simplex virus is an HSV-1. In some embodiments, the HSV-1 is attenuated. In some embodiments, the HSV-1 is replication defective. In some embodiments, the HSV-1 is replication competent. In some embodiments, the HSV-1 has been engineered to reduce or eliminate expression of one or more HSV-1 genes (*e.g.,* one or more toxic HSV-1 genes). In some embodiments, the HSV-1 has reduced cytotoxicity as compared to a corresponding wild-type HSV-1. In some embodiments, the HSV-1 is not oncolytic.

[0106] In some embodiments, the herpes simplex virus has been modified to alter its tissue tropism relative to the tissue tropism of an unmodified, wild-type herpes simplex virus. In some embodiments, the herpes simplex virus comprises a modified envelope. In some embodiments, the modified envelope comprises one or more (*e.g.,* one or more, two or more, three or more, four or more, *etc.*) mutant herpes simplex virus glycoproteins. Examples of herpes simplex virus glycoproteins may include, but are not limited to, the glycoproteins gB, gC, gD, gH, and gL. In some embodiments, the modified envelope alters the herpes simplex virus tissue tropism relative to a wild-type herpes simplex virus.

[0107] In some embodiments, the transduction efficiency (*in vitro* and/or *in vivo*) of a virus of the present disclosure (*e.g.,* a herpes virus) for one or more target cells (*e.g.,* one or more cells of the respiratory tract) is at least about 25%. For example, the transduction efficiency of the virus for one or more target cells may be at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, at least about 99.5%, or more. In some embodiments, the virus is a herpes simplex virus and the transduction efficiency of the virus for one or more target cells (*e.g.,* one or more cells of the respiratory tract) is about 85% to about 100%. In some embodiments, the virus is a herpes simplex virus and the transduction efficiency of the virus for one or more target cells (*e.g.,* one or more cells of the respiratory tract) is at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100%. Methods of measuring viral transduction efficiency *in vitro* or *in vivo* are well known to one of ordinary skill in the art, including, for example, qPCR analysis, deep sequencing, western blotting,

fluorometric analysis (such as fluorescent *in situ* hybridization (FISH), fluorescent reporter gene expression, immunofluorescence, FACS), *etc.*

V. Pharmaceutical Compositions and Formulations

[0108] Certain aspects of the present disclosure relate to pharmaceutical compositions or formulations comprising any of the recombinant nucleic acids (*e.g.,* a recombinant herpes virus genome) and/or viruses (*e.g.,* a herpes virus comprising a recombinant genome) described herein (such as a herpes simplex virus comprising a recombinant herpes simplex virus genome), and a pharmaceutically acceptable excipient or carrier.

[0109] In some embodiments, the pharmaceutical composition or formulation comprises any one or more of the viruses (*e.g.,* herpes viruses) described herein. In some embodiments, the pharmaceutical composition or formulation comprises from about 10^4 to about 10^{12} plaque forming units (PFU)/mL of the virus. For example, the pharmaceutical composition or formulation may comprise from about 10^4 to about 10^{12}, about 10^5 to about 10^{12}, about 10^6 to about 10^{12}, about 10^7 to about 10^{12}, about 10^8 to about 10^{12}, about 10^9 to about 10^{12}, about 10^{10} to about 10^{12}, about 10^{11} to about 10^{12}, about 10^4 to about 10^{11}, about 10^5 to about 10^{11}, about 10^6 to about 10^{11}, about 10^7 to about 10^{11}, about 10^8 to about 10^{11}, about 10^9 to about 10^{11}, about 10^{10} to about 10^{11}, about 10^4 to about 10^{10}, about 10^5 to about 10^{10}, about 10^6 to about 10^{10}, about 10^7 to about 10^{10}, about 10^8 to about 10^{10}, about 10^9 to about 10^{10}, about 10^4 to about 10^9, about 10^5 to about 10^9, about 10^6 to about 10^9, about 10^7 to about 10^9, about 10^8 to about 10^9, about 10^4 to about 10^8, about 10^5 to about 10^8, about 10^6 to about 108, about 10^7 to about 10^8, about 10^4 to about 10^7, about 10^5 to about 10^7, about 10^6 to about 10^7, about 10^4 to about 10^6, about 10^5 to about 10^6, or about 10^4 to about 10^5 PFU/mL of the virus. In some embodiments, the pharmaceutical composition or formulation comprises about 10^4, about 10^5, about 10^6, about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, or about 10^{12} PFU/mL of the virus.

[0110] Pharmaceutical compositions and formulations can be prepared by mixing the active ingredient(s) (such as a recombinant nucleic acid and/or a virus) having the desired degree of purity with one or more pharmaceutically acceptable carriers or excipients. Pharmaceutically acceptable carriers or excipients are generally nontoxic to recipients at the dosages and concentrations employed, and may include, but are not limited to: buffers (such as phosphate, citrate, acetate, and other organic acids); antioxidants (such as ascorbic acid and methionine); preservatives (such as octadecyldimethylbenzyl ammonium chloride,

benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol); amino acids (such as glycine, glutamine, asparagine, histidine, arginine, or lysine); low molecular weight (less than about 10 residues) polypeptides; proteins (such as serum albumin, gelatin, or immunoglobulins); polyols (such as glycerol, *e.g.,* formulations including 10% glycerol); hydrophilic polymers (such as polyvinylpyrrolidone); monosaccharides, disaccharides, and other carbohydrates (including glucose, mannose, or dextrins); chelating agents (such as EDTA); sugars (such as sucrose, mannitol, trehalose, or sorbitol); salt-forming counter-ions (such as sodium); metal complexes (such as Zn-protein complexes); and/or non-ionic surfactants (such as polyethylene glycol (PEG)). A thorough discussion of pharmaceutically acceptable carriers is available in REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Pub. Co., N.J. 1991).

[0111] In some embodiments, the pharmaceutical composition or formulation comprises one or more lipid (*e.g.,* cationic lipid) carriers. In some embodiments, the pharmaceutical composition or formulation comprises one or more nanoparticle carriers. Nanoparticles are submicron (less than about 1000 nm) sized drug delivery vehicles that can carry encapsulated drugs (such as synthetic small molecules, proteins, peptides, cells, viruses, and nucleic acid-based biotherapeutics) for rapid or controlled release. A variety of molecules (*e.g.,* proteins, peptides, recombinant nucleic acids, *etc.*) can be efficiently encapsulated in nanoparticles using processes well known in the art. In some embodiments, a molecule "encapsulated" in a nanoparticle may refer to a molecule (such as a virus) that is contained within the nanoparticle or attached to and/or associated with the surface of the nanoparticle, or any combination thereof. Nanoparticles for use in the compositions or formulations described herein may be any type of biocompatible nanoparticle known in the art, including, for example, nanoparticles comprising poly(lactic acid), poly(glycolic acid), PLGA, PLA, PGA, and any combinations thereof (*see e.g.,* Vauthier *et al.* Adv Drug Del Rev. (2003) 55: 519-48; US2007/0148074; US2007/0092575; US2006/0246139; US5753234; US7081483; and WO2006/052285).

[0112] In some embodiments, the pharmaceutically acceptable carrier or excipient may be adapted for or suitable for any administration route known in the art, including, for example, intravenous, intramuscular, subcutaneous, cutaneous, oral, intranasal, intratracheal, sublingual, buccal, topical, transdermal, intradermal, intraperitoneal, intraorbital, intravitreal, subretinal, transmucosal, intraarticular, by implantation, by inhalation, intrathecal,

intraventricular, and/or intranasal administration. In some embodiments, the pharmaceutically acceptable carrier or excipient is adapted for or suitable for oral, intranasal, intratracheal, and/or inhaled administration. In some embodiments, the pharmaceutically acceptable carrier or excipient is adapted for or suitable for inhaled administration. In some embodiments, the pharmaceutically acceptable carrier or excipient is adapted for or suitable for non-invasive inhaled administration. In some embodiments, the pharmaceutically acceptable carrier or excipient is adapted for or suitable for nebulization (*e.g.,* using a vibrating mesh nebulizer).

[0113] In some embodiments, the pharmaceutical composition or formulation is adapted for or suitable for any administration route known in the art, including, for example, intravenous, intramuscular, subcutaneous, cutaneous, oral, intranasal, intratracheal, sublingual, buccal, topical, transdermal, intradermal, intraperitoneal, intraorbital, intravitreal, subretinal, transmucosal, intraarticular, by implantation, by inhalation, intrathecal, intraventricular, or intranasal administration. In some embodiments, the pharmaceutical composition or formulation is adapted for or suitable for oral, intranasal, intratracheal, or inhaled administration. In some embodiments, the pharmaceutical composition or formulation is adapted for or suitable for inhaled administration. In some embodiments, the pharmaceutical composition or formulation is adapted for or suitable for non-invasive inhaled administration. In some embodiments, the pharmaceutical composition or formulation is adapted for or suitable for nebulization (*e.g.,* using a vibrating mesh nebulizer).

[0114] In some embodiments, the pharmaceutical composition or formulation further comprises one or more additional components. Examples of additional components may include, but are not limited to, binding agents (*e.g.,* pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*); fillers (*e.g.,* lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, *etc.*); lubricants (*e.g.,* magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*); disintegrants (*e.g.,* starch, sodium starch glycolate, *etc.*); wetting agents (*e.g.,* sodium lauryl sulphate, *etc.*); salt solutions; alcohols; polyethylene glycols; gelatin; lactose; amylase; magnesium stearate; talc; silicic acid; viscous paraffin; hydroxymethylcellulose; polyvinylpyrrolidone; sweetenings; flavorings; perfuming agents; colorants; moisturizers; sunscreens; antibacterial agents; agents able to stabilize polynucleotides or prevent their degradation, and the like. In some embodiments, the pharmaceutical composition or formulation comprises a phosphate buffer.

In some embodiments, the pharmaceutical composition or formulation comprises glycerol (*e.g.,* at about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, *etc.*). In some embodiments, the pharmaceutical composition or formulation comprises a phosphate buffer and glycerol. In some embodiments, the pharmaceutical composition or formulation comprises less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, or less than about 0.1% glycerol. In some embodiments, the pharmaceutical composition or formulation does not comprise glycerol.

[0115] Pharmaceutical compositions and formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.,* by filtration through sterile filtration membranes.

[0116] In some embodiments, any of the recombinant nucleic acids, viruses, and/or pharmaceutical compositions or formulations described herein may be used to deliver one or more polynucleotides encoding a CFTR polypeptide into one or more cells of a subject (*e.g.,* one or more CFTR-deficient cells, one or more cells harboring a *CFTR* gene mutation, one or more cells of the respiratory tract, *etc.*). In some embodiments, any of the recombinant nucleic acids, viruses, and/or pharmaceutical compositions or formulations described herein may be used in the treatment of a disease or condition that would benefit from the expression of a CFTR polypeptide (*e.g.,* a disease associated with a CFTR deficiency and/or a disease associated with a *CFTR* gene mutation). In some embodiments, any of the recombinant nucleic acids, viruses, and/or pharmaceutical compositions or formulations described herein may be used in the prevention or treatment of a chronic lung disease (such as cystic fibrosis, COPD, *etc.*). In some embodiments, any of the recombinant nucleic acids, viruses, and/or pharmaceutical compositions or formulations described herein may be used in the prevention or treatment of cystic fibrosis.

[0117] In some embodiments, any of the recombinant nucleic acids, viruses, and/or pharmaceutical compositions or formulations described herein may be used in the preparation of a medicament useful for delivering one or more polynucleotides encoding a CFTR polypeptide into one or more cells of a subject (*e.g.,* one or more CFTR-deficient cells, one or more cells harboring a *CFTR* gene mutation, one or more cells of the respiratory tract, *etc.*). In some embodiments, any of the recombinant nucleic acids, viruses, and/or pharmaceutical

compositions or formulations described herein may be used in the preparation of a medicament useful for the prevention or treatment of a disease or condition that would benefit from the expression of a CFTR polypeptide (*e.g.,* a disease associated with a CFTR deficiency and/or a disease associated with a *CFTR* gene mutation). In some embodiments, any of the recombinant nucleic acids, viruses, and/or pharmaceutical compositions or formulations described herein may be used in the preparation of a medicament useful for the prevention or treatment of a chronic lung disease (such as cystic fibrosis, COPD, *etc.*). In some embodiments, any of the recombinant nucleic acids, viruses, and/or pharmaceutical compositions or formulations described herein may be used in the preparation of a medicament useful for the prevention or treatment of cystic fibrosis.

VI. Methods

[0118] Certain aspects of the present disclosure relate to enhancing, increasing, augmenting, and/or supplementing the levels of a CFTR polypeptide in one or more cells of a subject comprising administering to the subject any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the subject is a human. In some embodiments, the subject's genome comprises a mutation (*e.g.,* a loss-of-function mutation) in an endogenous *CFTR* gene (one or both copies). In some embodiments, the subject suffers from a chronic lung disease, *e.g.*, cystic fibrosis, COPD, *etc.* In some embodiments, the subject suffers from cystic fibrosis.

[0119] In some embodiments, administration of the recombinant nucleic acid, virus, medicament, and/or pharmaceutical composition or formulation to the subject increases CFTR levels (transcript or protein levels) by at least about 2-fold in one or more contacted or treated cells of the subject, as compared to the endogenous levels of CFTR in one or more corresponding untreated cells in the subject. For example, administration of the recombinant nucleic acid, virus, medicament, and/or pharmaceutical composition or formulation may increase CFTR levels (transcript or protein levels) by at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 250-fold, at least about 500-fold, at least about 750-fold, at least about 1000-fold, or more in one or more contacted or treated cells of the subject, as compared to the endogenous levels of CFTR in one or more corresponding untreated cells in

the subject. In some embodiments, the one or more contacted or treated cells are one or more cells of the respiratory tract (*e.g.,* one or more cells of the airway epithelia and/or one or more cells of the submucosal glands). Methods of measuring transcript or protein levels from a sample are well known to one of ordinary skill in the art, including, for example, qPCR, western blot, mass spectrometry, *etc.*

[0120] Other aspects of the present disclosure relate to a method of reducing cellular sodium levels in a subject in need thereof comprising administering to the subject any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the subject is a human. In some embodiments, the subject's genome comprises a mutation (*e.g.,* a loss-of-function mutation) in an endogenous *CFTR* gene (one or both copies). In some embodiments, the subject suffers from a chronic lung disease, *e.g.,* cystic fibrosis, COPD, *etc.* In some embodiments, the subject suffers from cystic fibrosis.

[0121] In some embodiments, administration of the recombinant nucleic acid, virus, medicament, and/or pharmaceutical composition or formulation to the subject decreases intracellular sodium levels by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99% or more in one or more contacted or treated cells, as compared to the intracellular sodium levels in one or more corresponding untreated cells in the subject. Methods of measuring intracellular sodium levels are generally known to one of ordinary skill in the art.

[0122] Other aspects of the present disclosure relate to a method of improving a measure of at least one respiratory volume in a subject in need thereof comprising administering to the subject any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the subject is a human. In some embodiments, the subject's genome comprises a mutation (*e.g.,* a loss-of-function mutation) in an endogenous *CFTR* gene (one or both copies). In some embodiments, the subject suffers from a chronic lung disease, *e.g.,* cystic fibrosis, COPD, *etc.*

[0123] In some embodiments, administration of the recombinant nucleic acid, virus, medicament, and/or pharmaceutical composition or formulation to the subject improves a measure of at least one respiratory volume by at least about 5%, at least about 10%, at least

about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99% or more as compared to at least one reference respiratory volume measured in the subject prior to treatment. Examples of suitable respiratory volumes that may be measured include, for example: Total Lung Capacity (TLC), the volume in the lungs at maximal inflation; Tidal Volume (TV), the volume of air moved into or out of the lungs during quiet breathing; Residual Volume (RV), the volume of air remaining in the lungs after a maximal exhalation; Expiratory Reserve Volume (ERV), the maximal volume of air that can be exhaled (above tidal volume) during a forceful breath out; Inspiratory Reserve Volume (ERV), the maximal volume of air that can be inhaled from the end-inspiratory position; Inspiratory Capacity (IC), the sum of IRV and TV; Inspiratory vital capacity (IVC), the maximum volume of air inhaled form the point of maximum expiration; Vital Capacity (VC), the volume of air breathed our after the deepest inhalation; Functional Residual Capacity (FRC), the volume in the lungs at the end-expiratory position; Forced vital capacity (FVC), the determination of the vital capacity from a maximally forced expiratory effort; Forced Expiratory Volume (time) (FEV_t), the volume of air exhaled under forced conditions in the first t seconds; Forced Inspiratory Flow (FIF), a specific measurement of the forced inspiratory curve; Peak Expiratory Flow (PEF), the highest forced expiratory flow measured with a peak flow meter; Maximal Voluntary Ventilation (MVV), the volume of air expired in a specific period during repetitive maximal effort; *etc.* Methods of measuring respiratory volumes are generally known to one of ordinary skill in the art.

[0124] Other aspects of the present disclosure relate to a method of reducing or preventing chronic bacterial infections in the lungs of a subject in need thereof comprising administering to the subject any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the subject is a human. In some embodiments, the subject's genome comprises a mutation (*e.g.,* a loss-of-function mutation) in an endogenous *CFTR* gene (one or both copies). In some embodiments, the subject suffers from a chronic lung disease, *e.g.,* cystic fibrosis, COPD, *etc.* In some embodiments, the subject suffers from cystic fibrosis. Direct and indirect methods of monitoring bacterial infections in the lungs, including improvements thereto, are known to one of ordinary skill in the art, including, for example, by performing: blood tests or cultures,

oximetry, arterial blood gas measurements, bronchoscopy, transtracheal mucus cultures, lung biopsies, thoracentesis, computed tomography scans, *etc.*

[0125] Other aspects of the present disclosure relate to a method of reducing, preventing, or treating chronic inflammation of the lungs of a subject in need thereof comprising administering to the subject any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the subject is a human. In some embodiments, the subject's genome comprises a mutation (*e.g.,* a loss-of-function mutation) in an endogenous *CFTR* gene (one or both copies). In some embodiments, the subject suffers from a chronic lung disease, *e.g.,* cystic fibrosis, COPD, *etc.* In some embodiments, the subject suffers from cystic fibrosis. Methods of measuring lung inflammation, including improvements thereto, are well known to one of ordinary skill in the art, including, for example, by measuring exhaled nitric oxide, determining the percentage of eosinophils in the sputum and/or blood, *etc.*

[0126] Other aspects of the present disclosure relate to a method of reducing, inhibiting, or treating progressive lung destruction in a subject in need thereof comprising administering to the subject any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the subject is a human. In some embodiments, the subject's genome comprises a mutation (*e.g.,* a loss-of-function mutation) in an endogenous *CFTR* gene (one or both copies). In some embodiments, the subject suffers from a chronic lung disease, *e.g.,* cystic fibrosis, COPD, *etc.* In some embodiments, the subject suffers from cystic fibrosis. Methods of measuring lung destruction are well known to one of ordinary skill in the art, including, for example, by the methods described by Saetta *et al.* (Am Rev Respir Dis. 1985 May;131(5):764-9).

[0127] Other aspects of the present disclosure relate to a method of providing prophylactic, palliative, or therapeutic relief to one or more signs or symptoms of cystic fibrosis in a subject in need thereof comprising administering to the subject an effective amount of any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the subject is a human. In some embodiments, the subject's genome comprises a mutation (*e.g.,* a loss-of-function mutation) in an endogenous *CFTR* gene (one or both copies).

[0128] Signs and symptoms of cystic fibrosis may include, without limitation: persistent cough that produces thick mucus; thick sticky mucus that builds up in the airways; wheezing; breathlessness; sinusitis; repeated lung infections; inflamed nasal passages; bronchiectasis;

nasal polyps; hemoptysis; pneumothorax; pancreatitis; recurring pneumonia; respiratory failure; and any combinations thereof

[0129] Other aspects of the present disclosure relate to a method of providing prophylactic, palliative, or therapeutic relief to one or more signs or symptoms of COPD in a subject in need thereof comprising administering to the subject an effective amount of any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the subject is a human. In some embodiments, the subject is a smoker or an ex-smoker.

[0130] Signs and symptoms of COPD may include, without limitation: shortness of breath; wheezing; chest tightness; excess mucus in the lungs; a chronic cough; cyanosis; frequent respiratory infections; and any combinations thereof.

[0131] The recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein may be administered by any suitable method or route known in the art, including, without limitation, orally, intranasally, intratracheally, sublingually, buccally, topically, rectally, via inhalation, transdermally, subcutaneously, intradermally, intravenously, intraarterially, intramuscularly, intracardially, intraosseously, intraperitoneally, transmucosally, vaginally, intravitreally, intraorbitally, subretinally, intraarticularly, peri-articularly, locally, epicutaneously, or any combinations thereof. The present disclosure thus encompasses methods of delivering any of the recombinant nucleic acids, viruses, medicaments, or pharmaceutical compositions or formulations described herein to an individual (*e.g.,* an individual having, or at risk of developing, a chronic lung disease such as cystic fibrosis).

[0132] In some embodiments, the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein are administered orally, intranasally, intratracheally, and/or via inhalation. Methods of delivering drugs to the lungs via oral, intranasal, intratracheal, and or inhaled routes of administration or generally known to one of ordinary skill in the art (*see e.g.,* Gardenhire *et al.* A Guide to Aerosol Delivery Devices for Respiratory Therapists, 4th Edition, American Association for Respiratory care, 2017; Patil *et al.* Pulmonary Drug Delivery Strategies: A Concise, Systematic Review, Lung India. 2012. 29(1):44-9; Marx *et al.* Intranasal Drug Administration – An Attractive Delivery Route for Some Drugs, 2015).

[0133] In some embodiments, the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations are delivered to the lungs by inhalation

of an aerosolized formulation. Inhalation may occur through the nose and/or the mouth of the subject. Exemplary devices for delivering the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations to the lung may include, without limitation, dry powder inhalers, pressurized metered dose inhalers, soft mist inhalers, nebulizers (*e.g.,* jet nebulizers, ultrasonic nebulizers, vibrating mesh nebulizers), colliding jets, extruded jets, surface wave microfluidic atomization, capillary aerosol generation, electrohydrodynamic aerosol devices, *etc.* (*see e.g.,* Carvalho and McConville. The function and performance of aqueous devices for inhalation therapy.(2016) Journal of Pharmacy and Pharmacology.

[0134] Liquid formulations may be administered to the lungs of a subject, *e.g.,* using a pressurized metered dose inhaler (pMDI). pMDIs generally include at least two components: a canister in which the liquid formulation is held under pressure in combination with one or more propellants, and a receptacle used to hold and actuate the canister. The canister may contain a single dose or multiple doses of the formulation. The canister may include a valve, typically a metering valve, from which the contents of the canister may be discharged. Aerosolized drug is dispensed from the pMDI by applying a force on the canister to push it into the receptacle, thereby opening the valve and causing the drug particles to be conveyed from the valve through the receptacle outlet. Upon discharge from the canister, the liquid formulation is atomized, forming an aerosol. pMDIs typically employ one or more propellants to pressurize the contents of the canister and to propel the liquid formulation out of the receptacle outlet, forming an aerosol. Any suitable propellants may be utilized, and may take a variety of forms, including, for example, a compressed gas or a liquified gas.

[0135] Liquid formulations may be administered to the lungs of a subject, *e.g.,* using a nebulizer. Nebulizers are liquid aerosol generators that convert the liquid formulation into mists or clouds of small droplets, often having diameters less than about 5 microns mass median aerodynamic diameter, which can be inhaled into the lower respiratory tract. The droplets carry the active agent(s) into the nose, upper airways, and/or deep lungs when the aerosol cloud is inhaled. Any type of nebulizer known in the art may be used to administer the formulation to a patient, including, without limitation, pneumatic (jet) nebulizers, electromechanical nebulizers (*e.g.,* ultrasonic nebulizers, vibrating mesh nebulizers, *etc.*), *etc.* Pneumatic (jet) nebulizers use a pressurized gas supply as a driving force for atomization of the liquid formulation. Compressed gas is delivered through a nozzle or jet to create a low-pressure field which entrains a surrounding liquid formulation and shears it into a thin film or

filaments. The film or filaments are unstable and break up into small droplets that are carried by the compressed gas flow into the inspiratory breath. Baffles inserted into the droplet plume screen out the larger droplets and return them to the bulk liquid reservoir. Electromechanical nebulizers use electrically generated mechanical force to atomize liquid formulations. The electromechanical driving force can be applied, for example, by vibrating the liquid formulation at ultrasonic frequencies, or by forcing the bulk liquid through small holes in a thin film. The forces generate thin liquid films or filament streams which break up into small droplets to form a slow-moving aerosol stream which can be entrained in an inspiratory flow. In some embodiments, the nebulizer is a vibrating mesh nebulizer. Examples of vibrating mesh nebulizers include, for example, the Phillips InnoSpire, the Aerogen Solo, the PARI eFlow, *etc.*

[0136] Liquid formulations may be administered to the lungs of a subject, *e.g.,* using an electrohydrodynamic (EHD) aerosol device. EHD aerosol devices use electrical energy to aerosolize liquid drug solutions or suspensions.

[0137] Dry powder formulations may be administered to the lungs of a subject, *e.g.,* using a dry powder inhaler (DPI). DPIs typically use a mechanism such as a burst of gas to create a cloud of dry powder inside a container, which can then be inhaled by the subject. In a DPI, the dose to be administered is stored in the form of a non-pressurized dry powder and, upon actuation of the inhaler, the particles of the powder are inhaled by the subject. In some cases, a compressed gas may be used to dispense the powder, similar to pMDIs. In some cases, the DPI may be breath actuated (an aerosol is created in precise response to inspiration). Typically, dry powder inhalers administer a dose of less than a few tens of milligrams per inhalation to avoid provocation of cough. Examples of DPIs include, for example, the Turbohaler® inhaler (AstraZeneca), the Clickhaler® inhaler (Innovata), the Diskus® inhaler (Glaxo), the EasyHaler® (Orion), the Exubera® inhaler (Pfizer), *etc.*

[0138] In some embodiments, the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations are administered once to the subject. In some embodiments, the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions are administered at least twice (*e.g.,* at least 2 times, at least 3 times, at least 4 times, at least 5 times, at least 10 times, *etc.*) to the subject. In some embodiments, at least about 1 hour (*e.g.,* at least about 1 hour, at least about 6 hours, at least about 12 hours, at least about 18 hours, at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7

days, at least about 15 days, at least about 20 days, at least about 30 days, at least about 40 days, at least about 50 days, at least about 60 days, at least about 70 days, at least about 80 days, at least about 90 days, at least about 100 days, at least about 120 days, *etc.*) pass between administrations (*e.g.,* between the first and second administrations, between the second and third administrations, *etc.*). In some embodiments, the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations are administered one, two, three, four, five or more times per day to the subject. In some embodiments, the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations are administered one, two, three, four, five or more times per month to the subject. In some embodiments, the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations are administered one, two, three, four, five or more times per year to the subject.

VII. Host cells

[0139] Certain aspects of the present disclosure relate to one or more host cells comprising any of the recombinant nucleic acids described herein. Any suitable host cell (prokaryotic or eukaryotic) known in the art may be used, including, for example: prokaryotic cells including eubacteria, such as Gram-negative or Gram-positive organisms, for example Enterobacteriaceae such as Escherichia (*e.g., E. coli*), Enterobacter, Erminia, Klebsiella, Proteus, Salmonella (*e.g., S. typhimurium*), Serratia (*e.g., S. marcescans*), and Shigella, as well as Bacilli such as *B. subtilis* and *B. licheniformis*; fungal cells (*e.g., S. cerevisiae*); insect cells (*e.g.,* S2 cells, etc.); and mammalian cells, including monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651), human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture), baby hamster kidney cells (BHK, ATCC CCL 10), mouse Sertoli cells (TM4), monkey kidney cells (CV1 ATCC CCL 70), African green monkey kidney cells (VERO-76, ATCC CRL-1587), human cervical carcinoma cells (HELA, ATCC CCL 2), canine kidney cells (MDCK, ATCC CCL 34), buffalo rat liver cells (BRL 3A, ATCC CRL 1442), human lung cells (W138, ATCC CCL 75), human liver cells (Hep G2, HB 8065), mouse mammary tumor (MMT 060562, ATCC CCL51), TRI cells, MRC 5 cells, FS4 cells, human hepatoma line (Hep G2), Chinese hamster ovary (CHO) cells, including DHFR" CHO cells, and myeloma cell lines such as NS0 and Sp2/0. In some embodiments, the host cell is a human or non-human primate cell. In some embodiments, the host cells are cells from a cell line. Examples of suitable host cells or cell lines may include,

but are not limited to, 293, HeLa, SH-Sy5y, Hep G2, CACO-2, A549, L929, 3T3, K562, CHO-K1, MDCK, HUVEC, Vero, N20, COS-7, PSN1, VCaP, CHO cells, and the like.

[0140] In some embodiments, the recombinant nucleic acid is a herpes simplex viral vector. In some embodiments, the recombinant nucleic acid is a herpes simplex virus amplicon. In some embodiments, the recombinant nucleic acid is an HSV-1 amplicon or HSV-1 hybrid amplicon. In some embodiments, a host cell comprising a helper virus is contacted with an HSV-1 amplicon or HSV-1 hybrid amplicon described herein, resulting in the production of a virus comprising one or more recombinant nucleic acids described herein. In some embodiments, the virus is collected from the supernatant of the contacted host cell. Methods of generating virus by contacting host cells comprising a helper virus with an HSV-1 amplicon or HSV-1 hybrid amplicon are known in the art.

[0141] In some embodiments, the host cell is a complementing host cell. In some embodiments, the complementing host cell expresses one or more genes that are inactivated in any of the viral vectors described herein. In some embodiments, the complementing host cell is contacted with a recombinant herpes virus genome (*e.g.,* a recombinant herpes simplex virus genome) described herein. In some embodiments, contacting a complementing host cell with a recombinant herpes virus genome results in the production of a herpes virus comprising one or more recombinant nucleic acids described herein. In some embodiments, the virus is collected from the supernatant of the contacted host cell. Methods of generating virus by contacting complementing host cells with a recombinant herpes simplex virus are generally described in WO2015/009952, WO2017/176336, WO2019/200163, WO2019/210219, and/or WO2020/006486.

VIII. Articles of Manufacture or Kits

[0142] Certain aspects of the present disclosure relate to an article of manufacture or a kit comprising any of the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations described herein. In some embodiments, the article of manufacture or kit comprises a package insert comprising instructions for administering the recombinant nucleic acid, virus, medicament, and/or pharmaceutical composition or formulation to treat a CFTR deficiency (*e.g.,* in a subject harboring homozygous *CFTR* loss-of-function gene mutations) and/or to provide prophylactic, palliative, or therapeutic relief of a one or more signs or symptoms of a chronic lung disease (such as cystic fibrosis or COPD). In some embodiments, the article or manufacture or kit

further comprises a device for administering (*e.g.,* aerosolizing) the recombinant nucleic acid, virus, medicament, and/or pharmaceutical composition or formulation. In some embodiments, the device is a nebulizer (*e.g.,* a vibrating mesh nebulizer).

[0143] Suitable containers for the recombinant nucleic acids, viruses, medicaments, and/or pharmaceutical compositions or formulations may include, for example, bottles, vials, bags, tubes, and syringes. The container may be formed from a variety of materials such as glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container comprises a label on, or associated with the container, wherein the label indicates directions for use. The article of manufacture or kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, inhalers, nebulizers, intranasal administration devices, a package insert, and the like.

[0144] The specification is considered to be sufficient to enable one skilled in the art to practice the present disclosure. Various modifications of the present disclosure in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

EXAMPLES

[0145] The present disclosure will be more fully understood by reference to the following examples. It should not, however, be construed as limiting the scope of the present disclosure. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art, and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: modified herpes simplex virus vectors encoding a human CFTR protein

[0146] To make modified herpes simplex virus genome vectors capable of expressing CFTR polypeptides in a target mammalian cell (such as cells of the lung), a herpes simplex virus genome (**FIG. 1A**) is first modified to inactivate one or more herpes simplex virus genes. Such modifications may decrease the toxicity of the genome in mammalian cells. Next, variants of these modified/attenuated recombinant viral constructs are generated such that they carry one or more polynucleotides encoding the desired CFTR polypeptide. These variants include: 1) a recombinant ΔICP4-modified HSV-1 genome comprising expression cassettes containing the coding sequence (*e.g.,* SEQ ID NO: 2) of a human CFTR polypeptide

(*e.g.*, SEQ ID NO: 5) under the control of a heterologous promoter integrated at each ICP4 locus (**FIG. 1B**); 2) a recombinant ΔICP4/ΔUL41-modified HSV-1 genome comprising expression cassettes containing the coding sequence of a human CFTR polypeptide under the control of a heterologous promoter integrated at each ICP4 locus (**FIG. 1C**); 3) a recombinant ΔICP4/ΔUL41-modified HSV-1 genome comprising an expression cassette containing the coding sequence of a human CFTR polypeptide under the control of a heterologous promoter integrated at the UL41 locus (**FIG. 1D**); 4) a recombinant ΔICP4/ΔICP22-modified HSV-1 genome comprising expression cassettes containing the coding sequence of a human CFTR polypeptide under the control of a heterologous promoter integrated at each ICP4 locus (**FIG. 1E**); 5) a recombinant ΔICP4/ΔICP22-modified HSV-1 genome comprising an expression cassette containing the coding sequence of a human CFTR polypeptide under the control of a heterologous promoter integrated at the ICP22 locus (**FIG. 1F**); 6) a recombinant ΔICP4/ΔUL41/ΔICP22-modified HSV-1 genome comprising expression cassettes containing the coding sequence of a human CFTR polypeptide under the control of a heterologous promoter integrated at each ICP4 locus (**FIG. 1G**); 7) a recombinant ΔICP4/ΔUL41/ΔICP22-modified HSV-1 genome comprising an expression cassette containing the coding sequence of a human CFTR polypeptide under the control of a heterologous promoter integrated at the UL41 locus (**FIG. 1H**); and 8) a recombinant ΔICP4/ΔUL41/ΔICP22-modified HSV-1 genome comprising an expression cassette containing the coding sequence of a human CFTR polypeptide under the control of a heterologous promoter integrated at the ICP22 locus (**FIG. 1I**)

[0147] These modified herpes simplex virus genome vectors are transfected into engineered cells that are modified to express one or more herpes virus genes. These engineered cells secrete into the supernatant of the cell culture a replication defective herpes simplex virus with the modified genomes packaged therein. The supernatant is then collected, concentrated, and sterile filtered through a 5 μm filter.

Example 2: construction and *in vitro* characterization of an HSV-1 vector encoding human CFTR in 2D cultures

[0148] Initial lung gene therapy clinical trials occurred in the early 1990s following the discovery of the genetic defect responsible for cystic fibrosis. Recombinant adenovirus was one of the early vectors tested for *CFTR* delivery; however, adeno-based vectors failed these trials mainly due to the paucity of viral receptors on the apical lung surface and the severity

of the host-immune response to repeated viral delivery. The other viral gene therapy vectors administered to CF patients were based on adeno-associated virus (numerous AAV serotypes have been tested in the CF clinical setting). Large repeat administration studies of AAV-based gene therapy vectors provided disappointing results in improving CF lung function in dosed patients. Much like adenovirus, recombinant AAV vectors do not efficiently infect the apical lung surface, and due to physical limitations of the size of encoded cargo, AAV vectors do not efficiently delivery full length human *CFTR*. Despite more than two decades of intensive effort, viral-based gene therapies have yet to help patients with CF (or any other obstructive lung disease).

[0149] At present, according to the US Cystic Fibrosis Foundation, there are no ongoing clinical trials of viral gene therapies in CF, and only two virus-based gene therapy vectors are in preclinical development (both of which are based on AAV, a vector that, as noted above, has already failed multiple clinical trials in CF patients). Instead, focus has shifted away from virus-based vectors to non-viral methods of *CFTR* delivery (*e.g.,* DNA plasmids or mRNAs complexed with liposomes). Unfortunately, these non-viral vectors have seen only limited success, due, at least in part, to the significant hurdles faced by product instability and/or inefficient delivery/transfection of liposomal formulations. All-in-all, over 25 clinical trials involving more than 470 patients testing viral and non-viral gene vectors have failed to show clinical benefit, largely due to inefficient gene transfer to target cells and host immune-mediated clearance after repeated exposure.

[0150] To this end, a recombinant herpes simplex virus type 1 (HSV-1) vector encoding full-length human CFTR (HSV-CFTR) was developed as a novel gene therapy for the treatment of CF patients. Without wishing to be bound by theory, it is believed that an HSV-based approach overcomes many of the hurdles experienced by other gene therapy vectors for CF, including the capacity to encode full-length human CFTR, the high efficiency of target cell transduction (HSV preferentially infects the apical membrane of polarized epithelial cells), the stability of the virus, and the established clinical safety of repeated administration of a product employing the same viral backbone as HSV-CFTR in the context of the highly inflammatory environment of wounded skin (ClinicalTrials.gov Identifier: NCT03536143). The following example describes experiments showing that this novel HSV-based gene therapy vector was capable of expressing functional, full-length human CFTR in cystic fibrosis patient-derived small airway epithelial cells (SAECs) in a dose-dependent manner.

[0151] HSV-CFTR was constructed as described in Example 1 above. Primary CF patient SAECs grown in 2D culture were left uninfected (mock) or were infected with HSV-CFTR at multiplicities of infection (MOIs) of 0.3, 1, or 3. Human *CFTR* expression was evaluated 48 hours post-infection in harvested cells by quantitative reverse transcription PCR (qRT-PCR). Codon-optimized *CFTR* transcripts were detected in infected primary CF SAECs at an MOI as low as 0.3, and appeared to show a dose-dependent increase in transgene expression up to an MOI of 3.0 (**FIG. 2**). Little-to-no exogenous *CFTR* RNA was observed in mock infected control samples, demonstrating specificity of the assay for the HSV-encoded human transgene.

[0152] CFTR protein expression in HSV-CFTR-infected primary CF SAECs was assessed via western blot analysis. GAPDH was used as a control to ensure consistent loading of samples. CF patient SAECs overexpressed human CFTR when infected with HSV-CFTR, as compared to mock-infected control cells (**FIG. 3**). Interestingly, while the endogenous CFTR protein in mock infected cells resolved as a single band slightly larger than 150 kDa (the predicted size of full-length human CFTR is 168 kDa), the exogenous CFTR protein expressed in HSV-CFTR-transduced cells appeared as a doublet of significantly larger size. Human CFTR is known to exist in three different forms depending on glycosylation status: (1) nonglycosylated; (2) core glycosylated; and (3) complex glycosylated, fully mature (Scanlin, 2001, *Respir Res,* 2(5), pp. 276-9). The appearance of the single lower molecular weight band in mock infected CF patient cells suggested that the endogenous (mutant) protein solely exists in the nonglycosylated form, indicative of an immature protein variant that does not properly traffic through the endoplasmic reticulum (ER) to the cell surface. In stark contrast, the appearance of the two larger forms of CFTR in HSV-CFTR infected cells revealed extensive post-translation modification of the human transgene, likely representing the core glycosylated and complex glycosylated variants of CFTR, suggesting proper maturation and trafficking of the exogenous protein through the ER.

[0153] CFTR protein expression and relative localization was next examined by immunofluorescence. Primary CF patient SAECs were transduced with HSV-CFTR at the indicated MOIs for 48 hours, and immunofluorescence staining for human CFTR was performed. A mock infected control sample was added to show baseline levels and cellular localization of the endogenous mutant CFTR protein in these diseased cells. When analyzed in the context of the control cells, the immunofluorescence data demonstrated that transduced SAECs displayed an HSV-CFTR dose-dependent increase in CFTR protein expression (**FIG.**

4A). When comparing the relative cellular localization of CFTR expressed in mock-infected vs. HSV-CFTR-infected CF patient SAECs (**FIG. 4B**), the CFTR expressed in uninfected cells appeared to be relegated to the perinuclear region (suggestive of entrapment and turnover in the ER), while CFTR was found throughout the cytoplasm and at the cell surface of HSV-CFTR transduced cells (indicative of proper maturation in, and trafficking through, the ER). This data was in agreement with the western blot data that suggested that the wild-type, HSV-CFTR-expressed CFTR was fully glycosylated while the endogenous, mutant CFTR was nonglycosylated (**FIG. 3**).

[0154] Finally, functionality of the HSV-CFTR-expressed human CFTR in infected CF patient SAECs was confirmed using a dihydrorhodamine 6G (dR6G) fluorescent dye uptake assay which was previously validated as a functional endpoint for virus-mediated CFTR restoration in 2D CF patient epithelial cell culture (Wersto, 1996, *Proc Natl Acad Sci USA,* 93(3), pp. 1167-72). Briefly, HSV-CFTR or mock-infected primary CF patient SAECs were incubated with dR6G-containing cell culture medium for 15 minutes, washed four times with PBS, lysed in RIPA buffer, and 526nm excitation/555nm emission fluorescence was read for each sample on a plate reader. dR6G is itself non-fluorescent, but is converted to the fluorescent compound rhodamine 6G upon cellular uptake and exposure to intracellular dehydrogenases, a process that depends on the presence of functional CFTR (Wersto, 1996, *Proc Natl Acad Sci USA,* 93(3), pp. 1167-72). A BCA assay was performed on each cell lysate to quantify total protein content, and relative fluorescence per µg total protein was calculated for each sample (**FIG. 5**). HSV-CFTR infection of primary CF patient SAECs caused a modest, dose-dependent increase in dR6G uptake as compared to mock infected controls, indicating that HSV-CFTR was capable of restoring CFTR function in these diseased primary epithelial cells.

Example 3: *in vitro* HSV-CFTR dose-ranging and pharmacology in 3D organotypic cultures using CF patient-derived organoids

[0155] Mutations in the *CFTR* gene are classified into one of six classes by the primary mechanism leading to CFTR malfunction. Mutations affecting synthesis and processing result in more severe disease because little-to-no protein reaches the cell surface; mutations that do not interfere with luminal trafficking but reduce CFTR-mediated anion efflux often lead to less severe symptoms due to the retention of some residual CFTR function at the apical membrane (Foundation, 2019, *2018 Annual Data Report,* Bethesda: Cystic Fibrosis

Foundation). Because *CFTR* mutations affect distinctive stages of protein synthesis and function, recent drug development efforts have focused on small molecule modulator therapies targeting a specific source of the protein's defect. For example, ivacaftor, subclassified as a CFTR protein "potentiator", augments chloride secretion of membranal CFTR (providing clinical benefit for persons with specific class III and IV CFTR gating and conductance mutations), while elexacaftor, subclassified as a CFTR protein "corrector", acts by facilitating the proper folding and cellular processing of CFTR that would otherwise be degraded by the endoplasmic reticulum's quality control pathway (providing clinical benefit for persons with specific class II CFTR trafficking mutations) (Clancy, 2019, *Am J Respir Crit Care Med,* 186(7), pp. 593-7). While recent FDA approval of four of these modulator therapies has been a boon to CF patients harboring the specific mutations responsive to these drugs, these modulators only treat a subset of the CF population. In particular need for effective drug intervention are patients harboring class I mutations (responsible for ~10% of CF cases worldwide), encompassing frameshift, splicing, and nonsense mutations that result in severely reduced or absent CFTR expression, as these patients suffer from the harshest and deadliest forms of CF (Wilschanski, 2012, *Front Pharmacol,* 20(3), pp. 1-3).

[0156] Due to a lack of adequate CF animal models, efficacy studies in air-liquid-interface-differentiated bronchial epithelial cells derived from CF patient lung explant materials have been used for some drug development efforts following proof-of-concept experimentation in heterologous 2D cell systems (Neuberger, 2011, *Methods Mol Biol,* 741(1), pp. 39-54) (Randell, 2011, *Methods Mol Biol,* 742(1), pp. 285-310). However, the limited availability of lung explant tissues and the invasive procedures necessary to obtain bronchial cells from CF patients without end-stage disease has led to development of 3D organotypic systems derived from "easy access" tissues harvested from *CFTR* mutant patients, for testing novel therapeutics to treat CF. One such technology, using a forskolin-induced swelling (FIS) assay, employs CF patient-derived intestinal organoids (PDOs) to study CFTR protein function alone or in response to pharmaceutical intervention (Dekkers, 2013, *Nat Med,* 19(7), pp. 939-45), and has proven to be a breakthrough in CF drug development. When exposed to forskolin, organoids rapidly increase their cyclic AMP content, which in turn results in the opening of the CFTR channel. Organoids derived from biopsies taken from healthy individuals swell as a consequence of ion and water transport into the organoid lumen mediated by CFTR, while organoids derived from CFTR mutant patient biopsies (or wild-type organoids exposed to specific pharmacological inhibition of

CFTR protein function) have reduced or completely inhibited swelling capacity (Boj, 2017, *J Vis Exp,* 120(1), p. e55159). Use of CF PDOs allows for the quantitative measure of CFTR protein function (via detection of organoid swelling) upon treatment with novel therapeutics, and positive results from this 3D organotypic system have been shown to directly correlate with clinical benefit, including both changes in pulmonary responses and sweat chloride concentration in treated CF patients (Berkers, 2019, *Cell Rep,* 26(7), pp. 1701-1708).

[0157] The following example describes experiments showing that the recombinant HSV-1 vector HSV-CFTR, characterized in Example 2 above, was capable of rescuing the cystic phenotype of CF PDOs, irrespective of the underlying *CFTR* mutation.

[0158] HSV-CFTR's ability to restore functional CFTR expression was tested in clinically relevant 3D organotypic cultures using intestinal organoids derived from four different CF patients; (1) a female patient homozygous for an F508del *CFTR* mutation (class II mutation), (2) a male patient also homozygous for the F508del mutation, (3) a female patient homozygous for a G542X nonsense *CFTR* mutation (class I mutation), and (4) a female patient homozygous for a W1282X nonsense *CFTR* mutation (class I mutation). To assess CFTR activity in transduced organoids, organoid morphology and size were assessed 24- or 48-hours post-infection, and a FIS assay was conducted as described previously (Boj, 2017, *J Vis Exp,* 120(1), p. e55159). For efficient infection of the CF organoids, the organoids were sheared into small fragments, incubated in solution with HSV-CFTR at the indicated MOIs for 1 hour, and seeded in 96-well clear bottom plates for analysis. The FIS assay was conducted 24- or 48-hours after seeding, as described in more detail below.

[0159] First, the G542X/G542X PDO was infected at MOIs of 10, 20, and 40 to evaluate both the vector's impact on organoid swelling and cell viability. Intestinal organoids derived from a healthy patient were plated in parallel as a comparator. Surprisingly, HSV-CFTR-transduced organoids showed lumen formation and a clear cystic morphology mimicking wild-type PDOs 24 hours post-infection, suggesting full functional correction of the diseased phenotype by the engineered vector prior to the addition of forskolin (**FIG. 6A**). An mCherry-expressing HSV vector was used as a negative control to show that the alterations in PDO morphology observed in the HSV-CFTR treated samples were not due to a non-specific response to viral infection. Next, a FIS assay was performed 48 hours after infection. At t=0, before the addition of forskolin and subsequent activation of CFTR, HSV-CFTR-transduced organoids already possessed a significantly enlarged lumen area, as compared to vehicle-treated or mCherry-infected organoids, in agreement with the observations at 24 hours post-

infection (**FIG. 6B**). Interestingly, only a moderate increase in organoid swelling was observed 60 minutes after the addition of forskolin (t=60) in HSV-CFTR transduced organoids, likely due to these organoids already being close to their maximum swelling potential prior to forskolin exposure (**FIG. 6C**). The G542X/G542X mutation can be (at least partially) corrected by exposure to the aminoglycoside geneticin (G418) that allows for translational readthrough of the nonsense mutation, and G418 was included in this assay as a positive control. While the G542X/G542X PDOs swelled in the presence of G418 at t=60, the average organoid size in these positive control samples were significantly smaller than those of HSV-CFTR-exposed PDOs (**FIGS. 6B and 6C**). Slight-to-moderate toxicity of the vector in the G542X/G542X PDOs was observed 48 hours after infection when HSV-CFTR was used at an MOI of 20 or 40, and toxicity at an MOI≥20 is likely causative of the diminished capacity for swelling observed in these organoids, as compared to the samples infected at an MOI of 10. However, even though a cytotoxic effect at high MOIs was observed, the treated organoids still outperformed the positive small molecule control.

[0160] Because HSV-CFTR corrected diseased organoids to the wild-type morphology (large cystic lumen) at all tested MOIs within 24 hours, and the higher HSV-CFTR doses appeared to negatively impact the organoids in the swelling assays, the three remaining cystic fibrosis PDOs were tested at lower HSV-CFTR doses (MOIs of 1, 5, and 10) and were analyzed via FIS assay 24 hours post-infection. First, HSV-CFTR was tested in PDOs derived from a patient that is homozygous for the F508del mutation of CFTR. F508del is the most common mutation in cystic fibrosis patients; at least one copy of this allele is found in approximately 85% of CF patients worldwide, and F508del accounts for about 70% of CFTR loss-of-function mutations (Maiuri, 2015, *Ann Transl Med,* 3(Supple 1), p. S24). The majority of the tested F508del organoid cultures showed a cystic (wild-type) morphology 24 hours after infection with HSV-CFTR, even at the lowest dose tested (MOI of 1). The average size of F508del organoids treated with HSV-CFTR was significantly increased compared to vehicle control or mCherry-infected organoids prior to forskolin addition (**FIG. 7A**). No significant change in average organoid size was detected after forskolin addition in HSV-CFTR-transduced samples, as these organoids are believed to already be at or near their maximal swelling capacity, *i.e.,* "pre-swollen" (**FIG. 7B**). Importantly, functional correction of the CFTR defect in F508del organoids was found to be similar between the HSV-CFTR-treated organoids prior to forskolin treatment and the positive control Orkambi®-exposed organoids 60 minutes after forskolin treatment (**FIG. 7A** vs. **FIG. 7B**). Orkambi® is a

combination therapy of lumacaftor/ivacaftor that is FDA-approved for the treatment of CF patients aged 2 years and older who are homozygous for the F508del mutation. No apparent cytotoxicity attributable to the vector was observed at any of the MOIs tested.

[0161] Next, organoids derived from a patient homozygous for a second nonsense *CFTR* mutation (W1282X) were infected with HSV-CFTR, and organoid size was quantified before and after forskolin addition. In agreement with the data presented in **FIG. 6** above, HSV-CFTR efficiently restored the wild-type cystic phenotype and increased the average organoid size 24 hours post-infection in the W1282X/W1282X nonsense CFTR PDOs prior to forskolin addition (**FIG. 8A**). Again, HSV-CFTR at an MOI as low as 1 appeared to correct the diseased morphology both before and after forskolin addition (**FIGS. 8A and 8B**). G418 was also included in these experiments; however, the W1282X/W1282X PDOs were found not to respond to this readthrough aminoglycoside, so no positive control could be included in this experiment (as no effective therapy currently exists for all nonsense *CFTR* mutations). This data suggested that HSV-CFTR could restore CFTR function in both G418-responsive and G418-non-responsive CFTR null patient samples.

[0162] Finally, organoids from a second F508del homozygous patient were tested. PDOs infected with HSV-CFTR had a slightly increased average size compared to vehicle-treated organoids, but this difference was not statistically significant (**FIGS 9A and 9B**).

[0163] The data from these studies revealed that transduction of intestinal CF organoids with HSV-CFTR resulted in a striking alteration of organoid morphology, from a compact budding CF phenotype to a cystic organoid phenotype containing a well-defined lumen exhibiting wild-type characteristics, within 24 hours of infection at MOIs ranging from 1 to 40. This "pre-swollen" wild-type phenotype was quantitatively demonstrated by measuring total organoid size, before the addition of forskolin and resulting activation of CFTR, in comparison to multiple negative controls. Due to the "pre-swollen" nature of HSV-CFTR-transduced organoids, the capacity for forskolin to stimulate further swelling was limited. The observation of a corrected cystic morphology in CF organoids exposed to low doses of HSV-CFTR suggested that high levels of exogenous wild-type CFTR expressed in a minority of cells was sufficient to establish disease correction, indicating a "dominant" effect of this therapeutic modality. One F508del organoid showed slightly less efficient restoration of the wild-type phenotype as compared to the other examined CF organoid cultures; however, a cystic morphology was observed in all CF organoids infected with HSV-CFTR at an MOI of 5 or higher. The differences observed between the various CF intestinal organoid cultures

were most likely due to slight alterations of their proliferative or differentiation status at the time of infection, and thus, it is unlikely that the *CFTR* genotype itself contributed meaningfully to the efficiency of HSV-CFTR transduction or functional CFTR expression. Put another away, HSV-CFTR corrected the CF diseased phenotype irrespective of the underlying *CFTR* mutation in this clinically translatable 3D organotypic system.

[0164] Taken together, the data provided in these Examples indicate that HSV-CFTR capably infected relevant airway epithelia, efficiently produced functional human CFTR, and molecularly corrected multiple *CFTR* defects without significant toxicity. Without wishing to be bound by theory, it is believed that these studies represent the first instance of experimental validation of an attenuated HSV-based gene therapy vector for delivering full-length functional human CFTR, supporting the application of HSV-CFTR as a novel, broadly applicable gene therapy for the treatment of CF.

Example 4: proof-of-concept *in vivo* administration of an inhaled HSV-based vector

[0165] The following example describes a proof-of-concept *in vivo* study examining the feasibility of administering an HSV-based vector to the trachea and/or lungs of immunocompetent animals after intranasal or intratracheal administration of the virus.

[0166] All procedures conducted in this example were in compliance with applicable animal welfare acts and were approved by the local Institutional Animal Care and Use Committee (IACUC). 10 five- to six-week old C57BL/6 mice were used in the study, five of which received either HSV-mCherry (described above) or vehicle control by intratracheal administration, and five of which received HSV-mCherry or vehicle control by intranasal administration. Prior to experimental procedures, the animals were sedated with an intraperitoneal injection of a mix of telazol/dexdomitor, and ophthalmic ointment was applied to the eyes to prevent drying of the corneas.

[0167] For intratracheal administration, the neck of each mouse was shaved using an electric razor, and depilatory cream was applied to remove all remaining fur. The surgical area was then cleaned twice with 70% ethanol-soaked swabs, and the anesthetized mice were positioned onto an angled restraint stand. A small incision in the neck was performed using surgical scissors, and the thymus, platysma, and anterior tracheal muscles were moved out of the way in order to visualize and access the tracheal rings. A 25µL intratracheal injection of $4.9375x10^8$ plaque forming units (PFUs) of HSV-mCherry was administered to three animals, while a 25µL intratracheal injection of vehicle control was administered to two

animals, and each mouse was held in a hanging position until its breathing gradually returned to normal. The incision site was closed with simple stiches, individually knotted.

[0168] For intranasal administration, mice were anesthetized as described above, and were position onto an angled restraining stand. Three mice were each inoculated intranasally with $4.9375x10^8$ PFUs of virus formulated in 25µL (12.5µL per nostril). The rate of formulation release was adjusted to allow the mouse to inhale the inoculum, without forming bubbles, during the inspiration phase of breathing. Two mice were administered 25µL of vehicle control using the same procedure. After administration, animals were held in a hanging position until breathing returned to normal.

[0169] All animals were allowed to recover from anesthesia, and were provided with water and food *ad libitum* until the time of sacrifice. 48 hours post-administration, mice were euthanized, and bronchoalveolar lavage (BAL) was performed in the left and right lungs using sterile saline. BAL fluid was collected, centrifuged, and the cell pellets were gathered. Next, the upper portions of the trachea were harvested and flash frozen in liquid nitrogen for nucleic acid quantification. The lungs (left lobe, right superior lobe, right middle lobe, and right inferior and post-caval lobes) were individually harvested and either flash frozen in liquid nitrogen for nucleic acid analysis or perfused in 4% neutral buffered formalin and embedded in paraffin for immunofluorescence analysis.

[0170] For immunofluorescence staining of paraffin embedded lung tissue, an Alexa Fluor® 488-conjugated pan cytokeratin antibody was used to detect epithelial cells (Invitrogen cat. no. 53-9003-82), and a rabbit anti-mCherry primary antibody (Abcam cat. no. ab213511) and Alexa Fluor® 594-conjugated secondary antibody (Abcam cat. no. ab150080) were used to detect infected cells. Tissue samples were mounted in mounting media containing DAPI to visualize nuclei.

[0171] Intranasal vs. intratracheal administration of HSV-mCherry resulted in similar levels of *mCherry* transcripts being detected in lung tissue of transduced animals (**FIG. 10A**). Interestingly, while little-to-no transgene transcripts were identified in the tracheas of intranasally-exposed mice, robust *mCherry* transcription was detected in the tracheas of intratracheally-exposed mice, with no statistically significant difference in transgene expression being observed between the lungs and tracheas of these invasively-treated animals. In addition, a greater average total cell count per mL of BAL fluid was observed in the intratracheally-administered animals (646,667 cells/mL and 393,333 cells/mL for intratracheal and intranasal administration, respectively), suggesting a greater influx of

inflammatory cells into the lungs after intratracheal administration of the HSV-based vector. Transgene protein expression in lung epithelial tissue was observed in both intranasally- (**FIG. 10B**) and intratracheally-exposed (**FIG. 10C**) animals dosed with HSV-mCherry, but not in the corresponding vehicle controls.

[0172] Taken together, this data indicates that an engineered HSV vector can be administered to the lungs of immunocompetent animals via multiple routes of administration, and further, that a non-invasive inhaled route of administration allows for similar levels of transgene expression in the lungs as a more direct, invasive route of administration, while concomitantly inducing less (inflammatory) cell invasion.

Example 5: nebulization of HSV-CFTR

[0173] The following example describes a study examining a non-invasive, nebulizer-based route of delivery for HSV-CFTR into the airways of wild-type and CFTR-deficient immunocompetent mice.

[0174] 16 mice are used in the study: 12 immunocompetent C57BL/6 animals and 4 immunocompetent gut-corrected CFTR-deficient animals. **Table 1** provides a summary of the study. 4 wild-type animals are administered HSV-CFTR via intranasal instillation, while the remaining animals are administered HSV-CFTR (or vehicle control) via nebulization (*e.g.*, employing a vibrating mesh nebulizer). 48 hours after dosing, animals are euthanized, BAL fluid is collected, and tissue samples along the respiratory tract and lungs are harvested, *i.e.*, the upper and lower trachea, the left and right bronchi, the left lung, and the right lung (superior, middle, inferior, and post-caval lobes, individually). Tissues from two animals/group are snap frozen in liquid nitrogen and a processed for nucleic acid analysis. Vector genomes/50ng total DNA are quantified in each tissue via qPCR analysis; human *CFTR* transcripts/50ng total RNA are quantified in each tissue via qRT-PCR analysis. Tissues from the remaining two animals/group are perfused and embedded in paraffin for immunofluorescence/immunohistochemistry. BAL fluid is processed to examine immune cell infiltration into the lungs.

Table 1 – Study Design

Group	Treatment	Route	n	Animals	Necropsy
1	Vehicle	Inhalation	4	C57BL/6	48 hours
2	HSV-CFTR	Intranasal instillation	4	C57BL/6	
3	HSV-CFTR	Inhalation	4	C57BL/6	
4	HSV-CFTR	Inhalation	4	*CFTR*^{tm1Unc}Tg(FABPCFTR)	

CLAIMS

What is claimed is:

1. A recombinant herpes virus genome comprising one or more polynucleotides encoding a cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide.

2. The recombinant herpes virus genome of claim 1, wherein the recombinant herpes virus genome is replication competent.

3. The recombinant herpes virus genome of claim 1, wherein the recombinant herpes virus genome is replication defective.

4. The recombinant herpes virus genome of any one of claims 1-3, wherein the recombinant herpes virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within one or more viral gene loci.

5. The recombinant herpes virus genome of any one of claims 1-4, wherein the recombinant herpes virus genome is selected from the group consisting of a recombinant herpes simplex virus genome, a recombinant varicella zoster virus genome, a recombinant human cytomegalovirus genome, a recombinant herpesvirus 6A genome, a recombinant herpesvirus 6B genome, a recombinant herpesvirus 7 genome, a recombinant Kaposi's sarcoma-associated herpesvirus genome, and any derivatives thereof.

6. The recombinant herpes virus genome of any one of claims 1-5, wherein the CFTR polypeptide is a human CFTR polypeptide.

7. The recombinant herpes virus genome of any one of claims 1-6, wherein the CFTR polypeptide comprises a 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 the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

8. The recombinant herpes virus genome of any one of claims 1-7, wherein the CFTR polypeptide comprises a 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 the amino acid sequence of SEQ ID NO: 5.

9. The recombinant herpes virus genome of any one of claims 1-8, wherein the recombinant herpes virus genome is a recombinant herpes simplex virus genome.

10. The recombinant herpes virus genome of claim 9, wherein the recombinant herpes simplex virus genome is a recombinant type 1 herpes simplex virus (HSV-1) genome, a recombinant type 2 herpes simplex virus (HSV-2) genome, or any derivatives thereof.

11. The recombinant herpes virus genome of claim 9 or claim 10, wherein the recombinant herpes simplex virus genome is a recombinant HSV-1 genome.

12. The recombinant herpes virus genome of any one of claims 9-11, wherein the recombinant herpes simplex virus genome has been engineered to reduce or eliminate expression of one or more toxic herpes simplex virus genes.

13. The recombinant herpes virus genome of any one of claims 9-12, wherein the recombinant herpes simplex virus genome comprises an inactivating mutation.

14. The recombinant herpes virus genome of claim 13, wherein the inactivating mutation is in a herpes simplex virus gene.

15. The recombinant herpes virus genome of claim 14, wherein the inactivating mutation is a deletion of the coding sequence of the herpes simplex virus gene.

16. The recombinant herpes virus genome of claim 14 or claim 15, wherein the herpes simplex virus gene is selected from the group consisting of Infected Cell Protein (ICP) 0, ICP4, ICP22, ICP27, ICP47, thymidine kinase (tk), Long Unique Region (UL) 41, and UL55.

17. The recombinant herpes virus genome of claim 16, wherein the recombinant herpes simplex virus genome comprises an inactivating mutation in one or both copies of the ICP4 gene.

18. The recombinant herpes virus genome of claim 16 or claim 17, wherein the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP22 gene.

19. The recombinant herpes virus genome of any one of claims 16-18, wherein the recombinant herpes simplex virus genome comprises an inactivating mutation in the UL41 gene.

20. The recombinant herpes virus genome of any one of claims 16-19, wherein the recombinant herpes simplex virus genome comprises an inactivating mutation in one or both copies of the ICP0 gene.

21. The recombinant herpes virus genome of any one of claims 16-20, wherein the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP27 gene.

22. The recombinant herpes virus genome of any one of claims 16-21, wherein the recombinant herpes simplex virus genome comprises an inactivating mutation in the ICP47 gene.

23. The recombinant herpes virus genome of any one of claims 16-22, wherein the recombinant herpes simplex virus genome comprises an inactivating mutation in the UL55 gene.

24. The recombinant herpes virus genome of any one of claims 9-23, wherein the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within one or both of the ICP4 viral gene loci.

25. The recombinant herpes virus genome of any one of claims 9-24, wherein the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the ICP22 viral gene locus.

26. The recombinant herpes virus genome of any one of claims 9-25, wherein the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the UL41 viral gene locus.

27. The recombinant herpes virus genome of any one of claims 9-26, wherein the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within one or both of the ICP0 viral gene loci.

28. The recombinant herpes virus genome of any one of claims 9-27, wherein the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the ICP27 viral gene locus.

29. The recombinant herpes virus genome of any one of claims 9-28, wherein the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the ICP47 viral gene locus.

30. The recombinant herpes virus genome of any one of claims 9-29, wherein the recombinant herpes simplex virus genome comprises the one or more polynucleotides encoding the CFTR polypeptide within the UL55 viral gene locus.

31. The recombinant herpes virus genome of any one of claims 1-30, wherein the recombinant herpes virus genome has reduced cytotoxicity when introduced into a target cell as compared to a corresponding wild-type herpes virus genome.

32. The recombinant herpes virus genome of claim 31, wherein the target cell is a human cell.

33. The recombinant herpes virus genome of claim 31 or claim 32, wherein the target cell is an airway epithelial cell.

34. The recombinant herpes virus genome of claim 31 or claim 32, wherein the target cell is a cell of the respiratory tract.

35. A herpes virus comprising the recombinant herpes virus genome of any one of claims 1-34.

36. The herpes virus of claim 35, wherein the herpes virus is replication competent.

37. The herpes virus of claim 35, wherein the herpes virus is replication defective.

38. The herpes virus of any one of claims 35-37, wherein the herpes virus has reduced cytotoxicity as compared to a corresponding wild-type herpes virus.

39. The herpes virus of any one of claims 35-38, wherein the herpes virus is selected from the group consisting of a herpes simplex virus, a varicella zoster virus, a human

cytomegalovirus, a herpesvirus 6A, a herpesvirus 6B, a herpesvirus 7, and a Kaposi's sarcoma-associated herpesvirus.

40. The herpes virus of any one of claims 35-39, wherein the herpes virus is a herpes simplex virus.

41. The herpes virus of claim 39 or claim 40, wherein the herpes simplex virus is a type 1 herpes simplex virus (HSV-1), a type 2 herpes simplex virus (HSV-2), or any derivatives thereof.

42. The herpes virus of any one of claims 39-41, wherein the herpes simplex virus is an HSV-1.

43. A pharmaceutical composition comprising the recombinant herpes virus genome of any one of claims 1-34 or the herpes virus of any one of claims 35-42 and a pharmaceutically acceptable excipient.

44. The pharmaceutical composition of claim 43, wherein the pharmaceutical composition is suitable for topical, transdermal, subcutaneous, intradermal, oral, intranasal, intratracheal, sublingual, buccal, rectal, vaginal, inhaled, intravenous, intraarterial, intramuscular, intracardiac, intraosseous, intraperitoneal, transmucosal, intravitreal, subretinal, intraarticular, peri-articular, local, or epicutaneous administration.

45. The pharmaceutical composition of claim 43 or claim 44, wherein the pharmaceutical composition is suitable for oral, intranasal, intratracheal, or inhaled administration.

46. The pharmaceutical composition of any one of claims 43-45, wherein the pharmaceutical composition is suitable for inhaled administration.

47. The pharmaceutical composition of any one of claims 43-46, wherein the pharmaceutical composition is suitable for non-invasive inhaled administration.

48. The pharmaceutical composition of any one of claims 43-47, wherein the pharmaceutical composition is suitable for use in a dry powder inhaler, a pressurized metered dose inhaler, a soft mist inhaler, a nebulizer, an electrohydrodynamic aerosol device, or any combinations thereof.

49. The pharmaceutical composition of any one of claims 43-48, wherein the pharmaceutical composition is suitable for use in a nebulizer.

50. The pharmaceutical composition of claim 49, wherein the nebulizer is a vibrating mesh nebulizer.

51. The pharmaceutical composition of any one of claims 43-50, wherein the pharmaceutical composition comprises a phosphate buffer.

52. The pharmaceutical composition of any one of claims 43-51, wherein the pharmaceutical composition comprises glycerol.

53. The pharmaceutical composition of any one of claims 43-52, wherein the pharmaceutical composition comprises a lipid carrier.

54. The pharmaceutical composition of any one of claims 43-53, wherein the pharmaceutical composition comprises a nanoparticle carrier.

55. A method of enhancing, increasing, augmenting, and/or supplementing the levels of a CFTR polypeptide in one or more cells of a subject, the method comprising administering to the subject an effective amount of the herpes virus of any one of claims 35-42 or the pharmaceutical composition of any one of claims 43-54.

56. The method of claim 55, wherein the one or more cells are one or more cells of the respiratory tract.

57. The method of claim 55 or claim 56, wherein the one or more cells are one or more airway epithelial cells or one or more cells of the submucosal glands.

58. A method of reducing or inhibiting progressive lung destruction in a subject in need thereof, the method comprising administering to the subject an effective amount of the herpes virus of any one of claims 35-42 or the pharmaceutical composition of any one of claims 43-54.

59. The method of any one of claims 55-58, wherein the subject suffers from a chronic lung disease.

60. The method of claim 59, wherein the chronic lung disease is cystic fibrosis or chronic obstructive pulmonary disease (COPD).

61. A method of providing prophylactic, palliative, or therapeutic relief of one or more signs or symptoms of cystic fibrosis in a subject in need thereof, the method comprising administering to the subject an effective amount of the herpes virus of any one of claims 35-42 or the pharmaceutical composition of any one of claims 43-54.

62. The method of claim 61, wherein the one or more signs or symptoms of cystic fibrosis are selected from the group consisting of a persistent cough that produces thick mucus, thick sticky mucus that builds up in the airways, wheezing, breathlessness, sinusitis, repeated lung infections, inflamed nasal passages, bronchiectasis, nasal polyps, hemoptysis, pneumothorax, pancreatitis, recurring pneumonia, respiratory failure, and any combinations thereof.

63. A method of providing prophylactic, palliative, or therapeutic relief of one or more signs or symptoms of COPD in a subject in need thereof, the method comprising administering to the subject an effective amount of the herpes virus of any one of claims 35-42 or the pharmaceutical composition of any one of claims 43-54.

64. The method of claim 63, wherein the one or more signs or symptoms of COPD are selected from the group consisting of shortness of breath, wheezing, chest tightness, excess mucus in the lungs, a chronic cough, cyanosis, frequent respiratory infections, and any combinations thereof.

65. The method of any one of claims 55-64, wherein the subject is a human.

66. The method of any one of claims 55-65, wherein the subject's genome comprises a loss-of-function mutation in a *CFTR* gene.

67. The method of any one of claims 55-66, wherein the herpes virus or pharmaceutical composition is administered orally, intranasally, intratracheally, or via inhalation to the subject.

68. The method of any one of claims 55-67, wherein the herpes virus or pharmaceutical composition is administered via inhalation to the subject.

69. The method of any one of claims 55-68, wherein the herpes virus or pharmaceutical composition is administered via non-invasive inhaled administration.

70. The method of any one of claims 55-69, wherein the herpes virus or pharmaceutical composition is administered using a dry powder inhaler, a pressurized metered dose inhaler, a soft mist inhaler, a nebulizer, or an electrohydrodynamic aerosol device.

71. The method of any one of claims 55-70, wherein the herpes virus or pharmaceutical composition is administered using a nebulizer.

72. The method of claim 71, wherein the nebulizer is a vibrating mesh nebulizer.

FIG. 1A

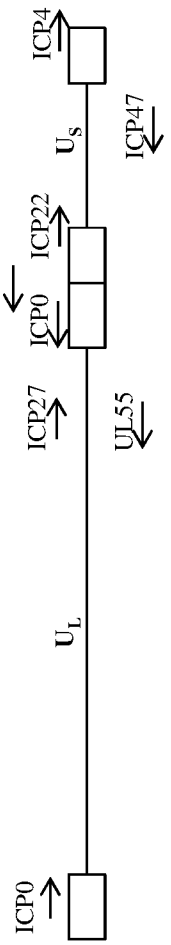

ICP0
U_L
ICP27
UL55
ICP4
ICP0
ICP22
U_S
ICP4
ICP47

FIG. 1B

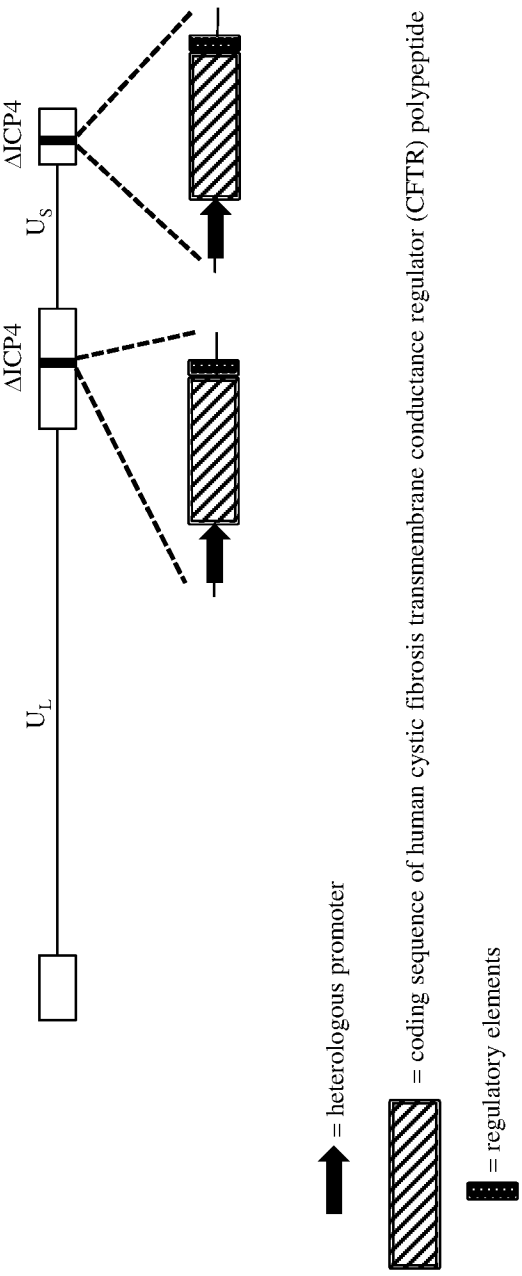

ΔICP4
ΔICP4
U_S
U_L
= heterologous promoter
= coding sequence of human cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide
= regulatory elements

FIG. 1C

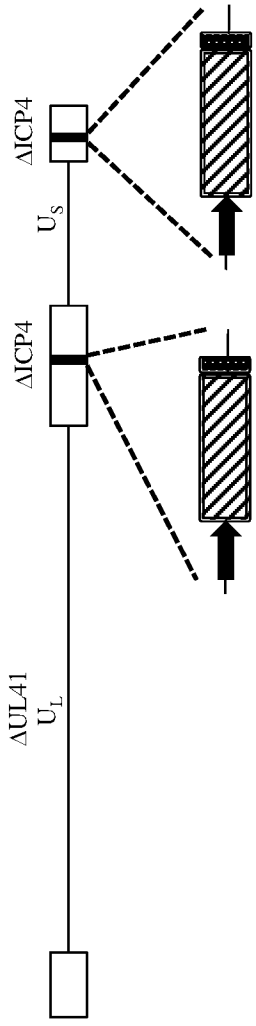

ΔICP4
U_S
ΔICP4
ΔUL41
U_L

FIG. 1D

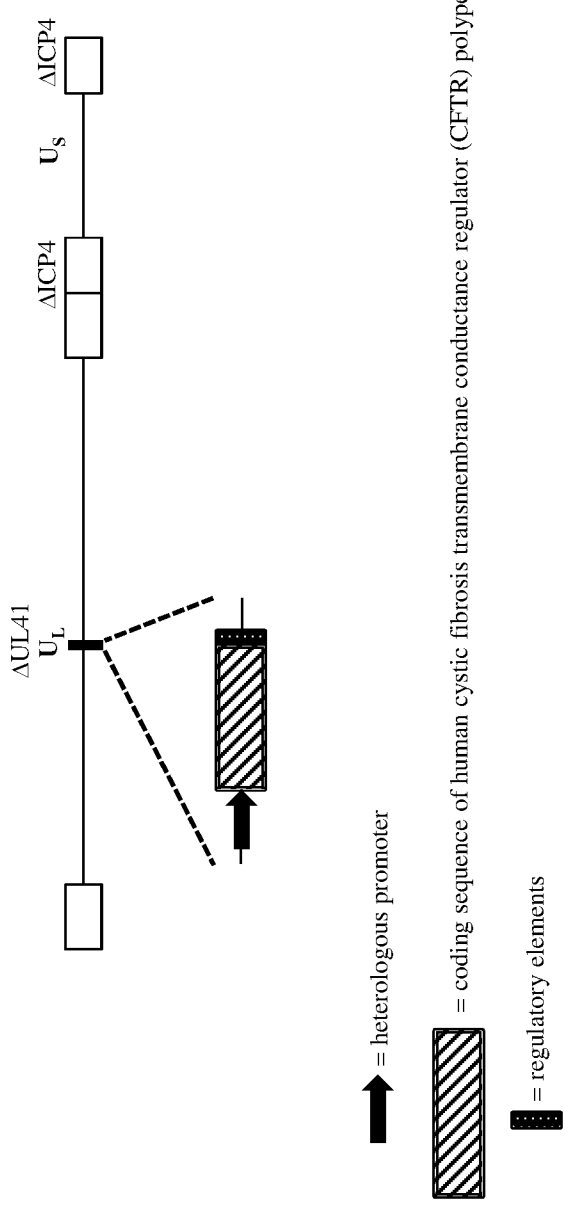

ΔICP4
U_S
ΔICP4
ΔUL41
U_L
= heterologous promoter
= coding sequence of human cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide
= regulatory elements

FIG. 1E

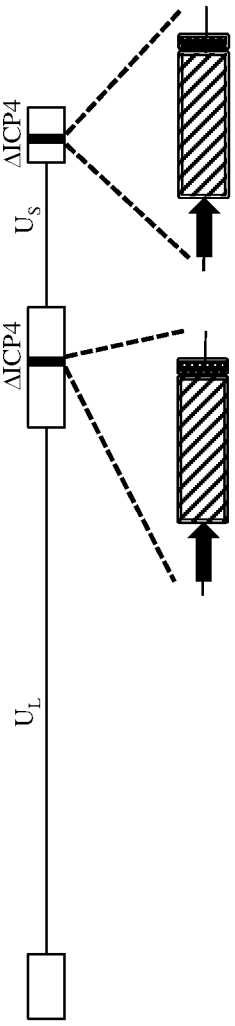
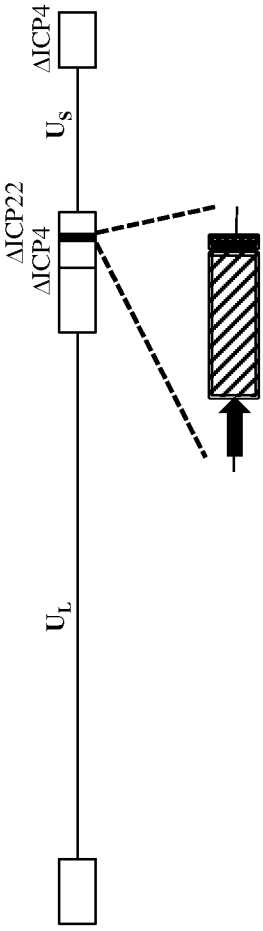


FIG. 1F



= heterologous promoter

= coding sequence of human cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide

= regulatory elements

FIG. 1G

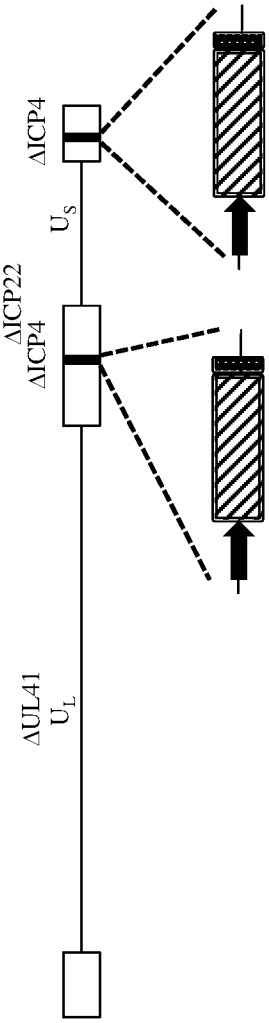
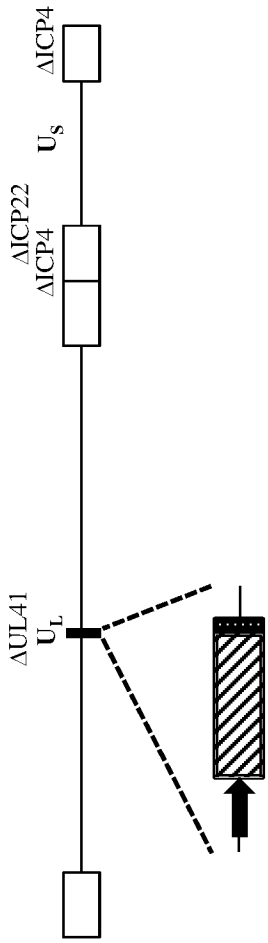

ΔICP4
U_S
ΔICP22
ΔICP4
ΔUL41
U_L

FIG. 1H


ΔICP4
U_S
ΔICP22
ΔICP4
ΔUL41
U_L

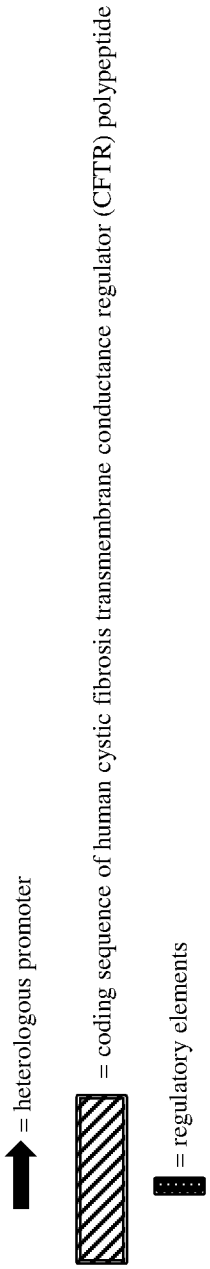

= heterologous promoter
= coding sequence of human cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide
= regulatory elements

FIG. 1I

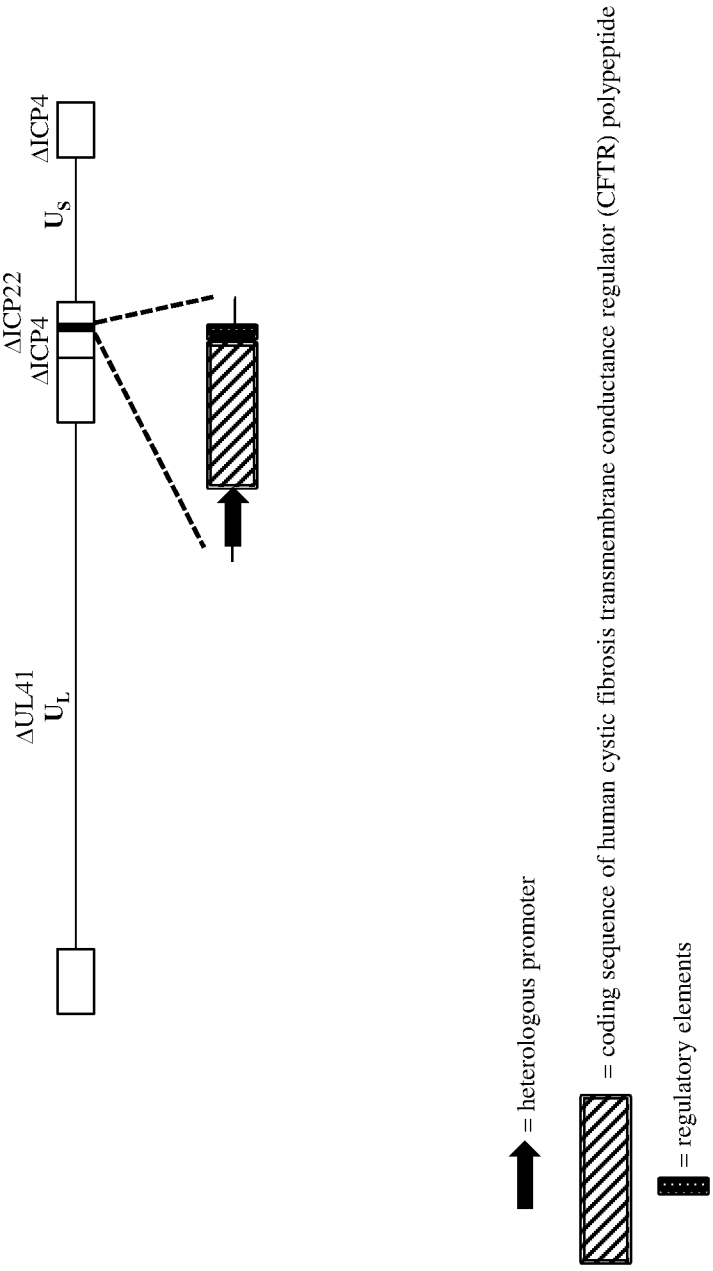

ΔICP4
U_S
ΔICP22
ΔICP4
ΔUL41
U_L
= heterologous promoter
= coding sequence of human cystic fibrosis transmembrane conductance regulator (CFTR) polypeptide
= regulatory elements

FIG. 2

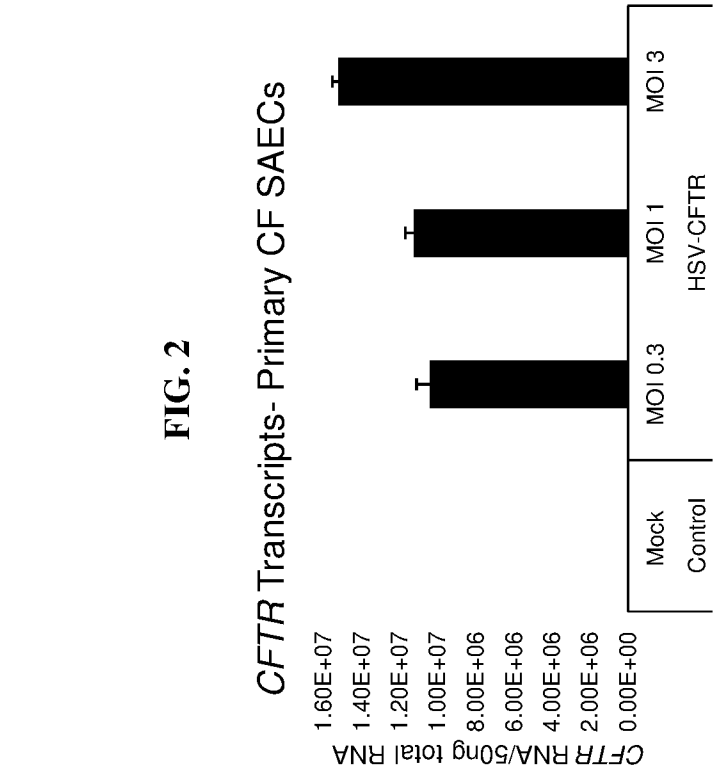

CFTR Transcripts- Primary CF SAECs
CFTR RNA/50ng total RNA
1.60E+07
1.40E+07
1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
Mock
Control
MOI 0.3
MOI 1
MOI 3
HSV-CFTR

FIG. 3

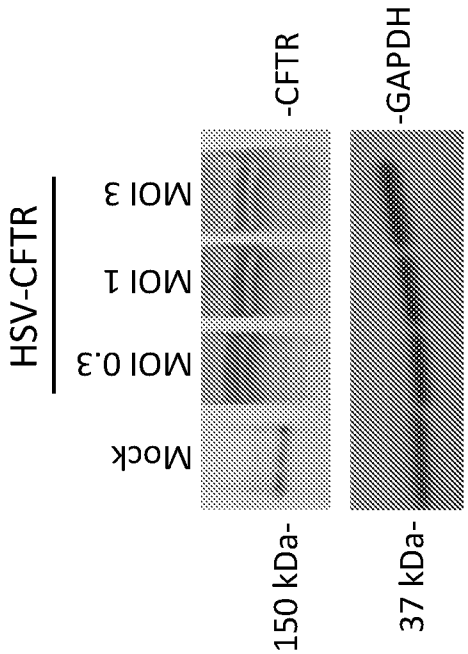

HSV-CFTR
Mock
MOI 0.3
MOI 1
MOI 3
150 kDa-
-CFTR
37 kDa-
-GAPDH

FIG. 4B

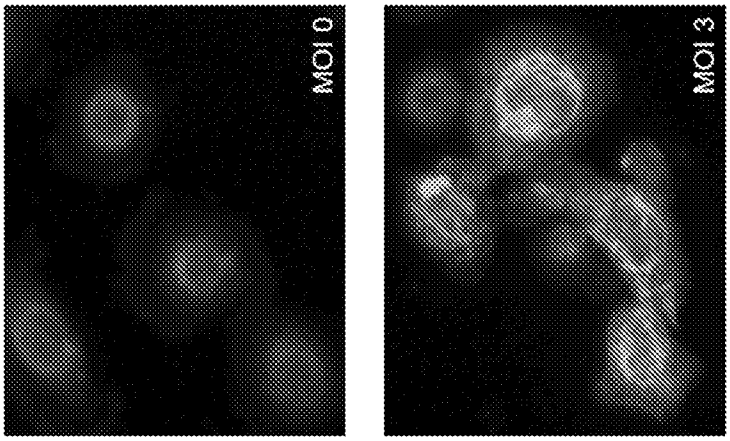


FIG. 4A

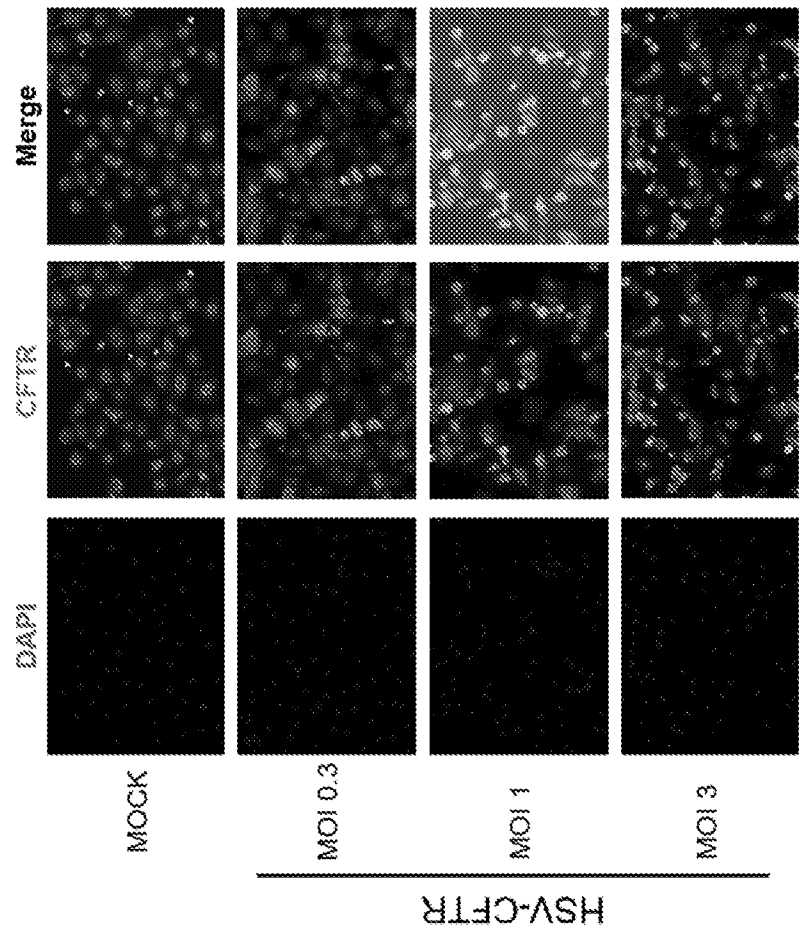


FIG. 5

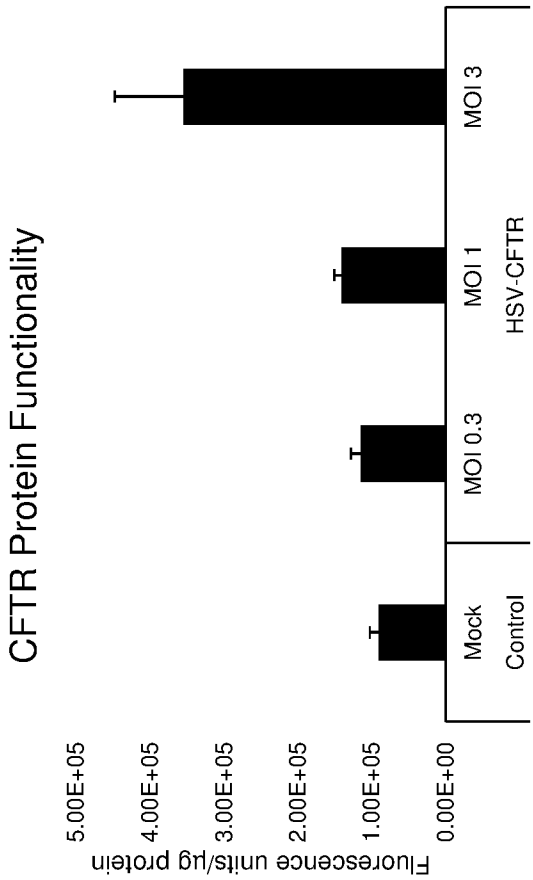

CFTR Protein Functionality
Fluorescence units/µg protein
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
Mock
MOI 0.3
MOI 1
MOI 3
Control
HSV-CFTR

FIG. 6A

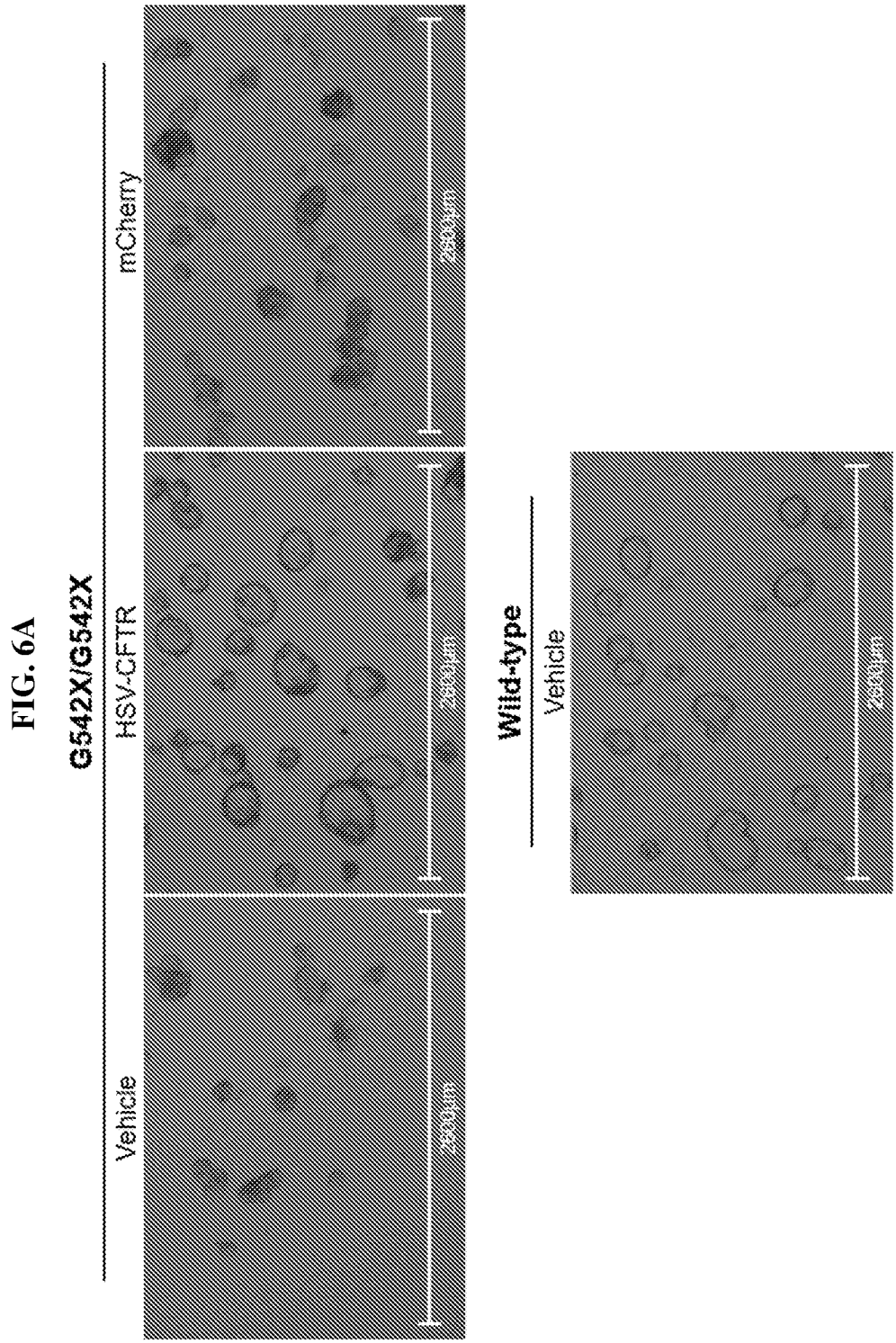

G542X/G542X
Vehicle
HSV-CFTR
mCherry
200μm
200μm
200μm
Wild-type
Vehicle
200μm

FIG. 6B

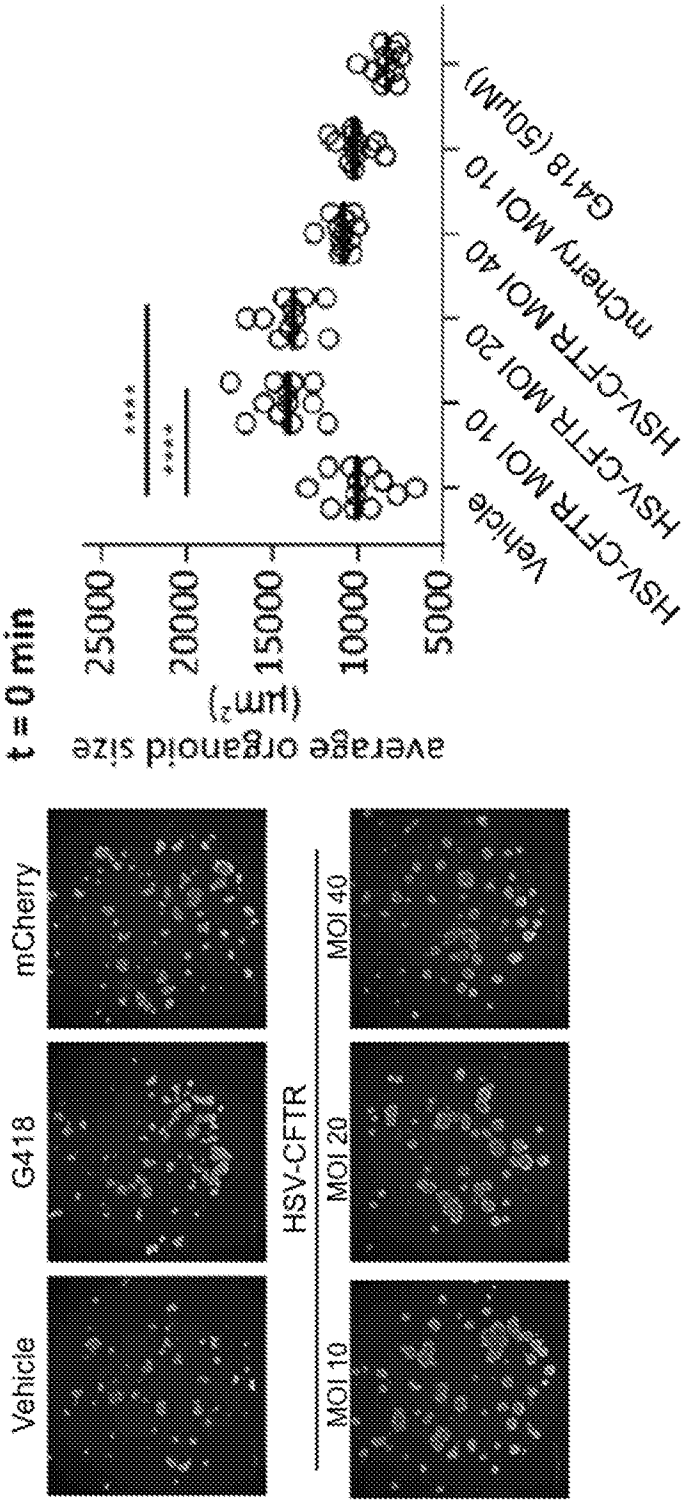

t = 0 min
Vehicle
G418
mCherry
HSV-CFTR
MOI 10
MOI 20
MOI 40
average organoid size
(μm³)
25000
20000
15000
10000
5000
****
****
Vehicle
HSV-CFTR MOI 10
HSV-CFTR MOI 20
HSV-CFTR MOI 40
mCherry MOI 10
G418 (50μM)

FIG. 6C

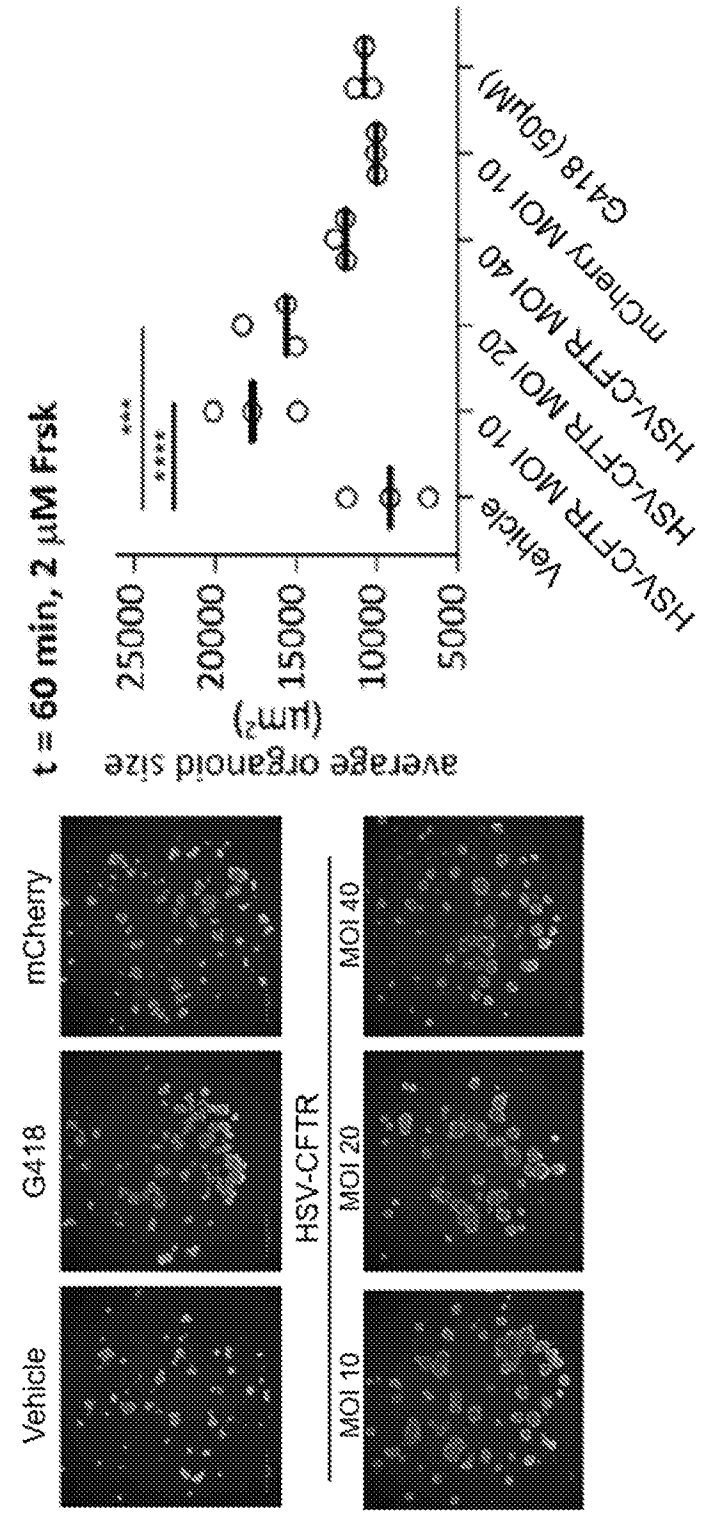

t = 60 min, 2 μM Frsk
average organoid size (μm²)
25000
20000
15000
10000
5000
***
****
Vehicle
HSV-CFTR MOI 10
HSV-CFTR MOI 20
HSV-CFTR MOI 40
mCherry MOI 10
G418 (50μM)
Vehicle
G418
mCherry
HSV-CFTR
MOI 10
MOI 20
MOI 40

FIG. 7A

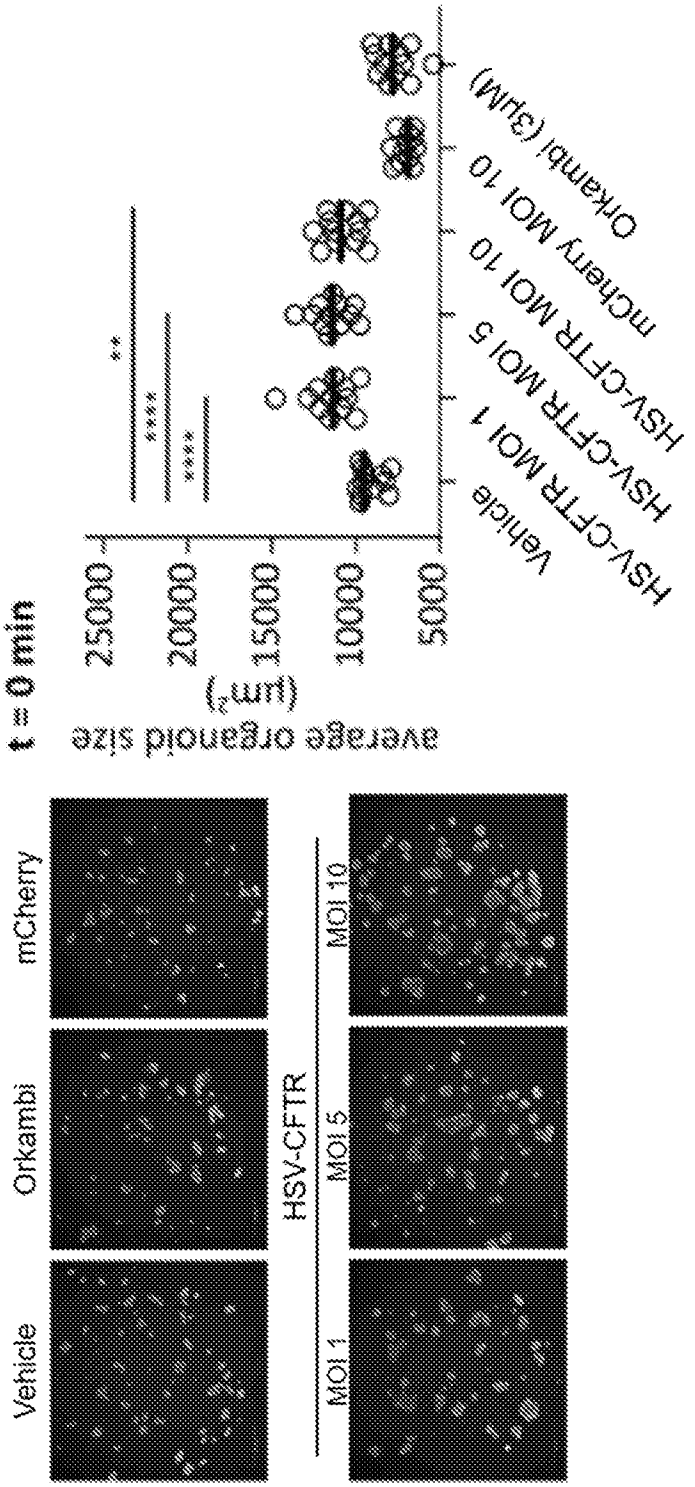

t = 0 min
Vehicle
Orkambi
mCherry
HSV-CFTR
MOI 1
MOI 5
MOI 10
average organoid size
(μm²)
25000
20000
15000
10000
5000
****
****
**
Vehicle
HSV-CFTR MOI 1
HSV-CFTR MOI 5
HSV-CFTR MOI 10
mCherry MOI 10
Orkambi (3μM)

FIG. 7B

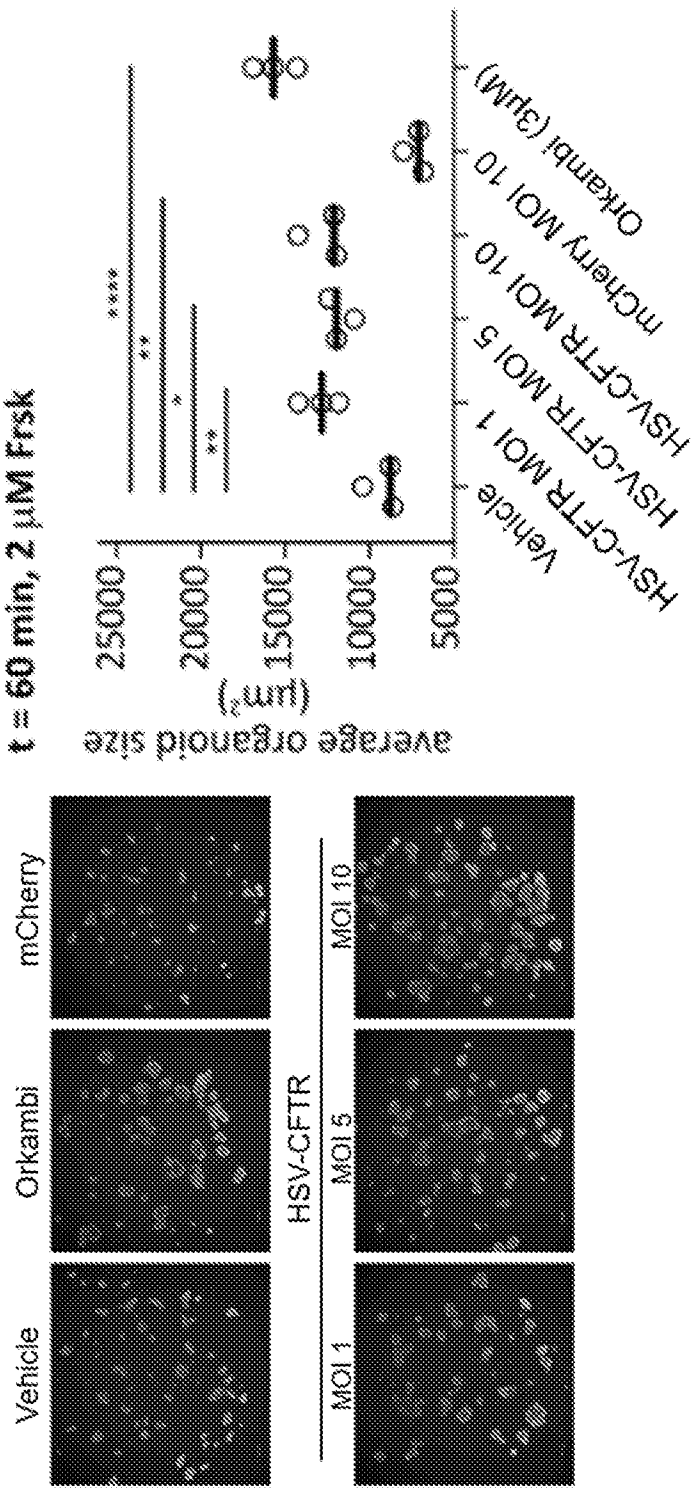

t = 60 min, 2 μM Frsk
average organoid size (μm³)
25000
20000
15000
10000
5000
****
**
*
**
Vehicle
HSV-CFTR MOI 1
HSV-CFTR MOI 5
HSV-CFTR MOI 10
mCherry MOI 10
Orkambi (3μM)
Vehicle
Orkambi
mCherry
HSV-CFTR
MOI 1
MOI 5
MOI 10

FIG. 8A

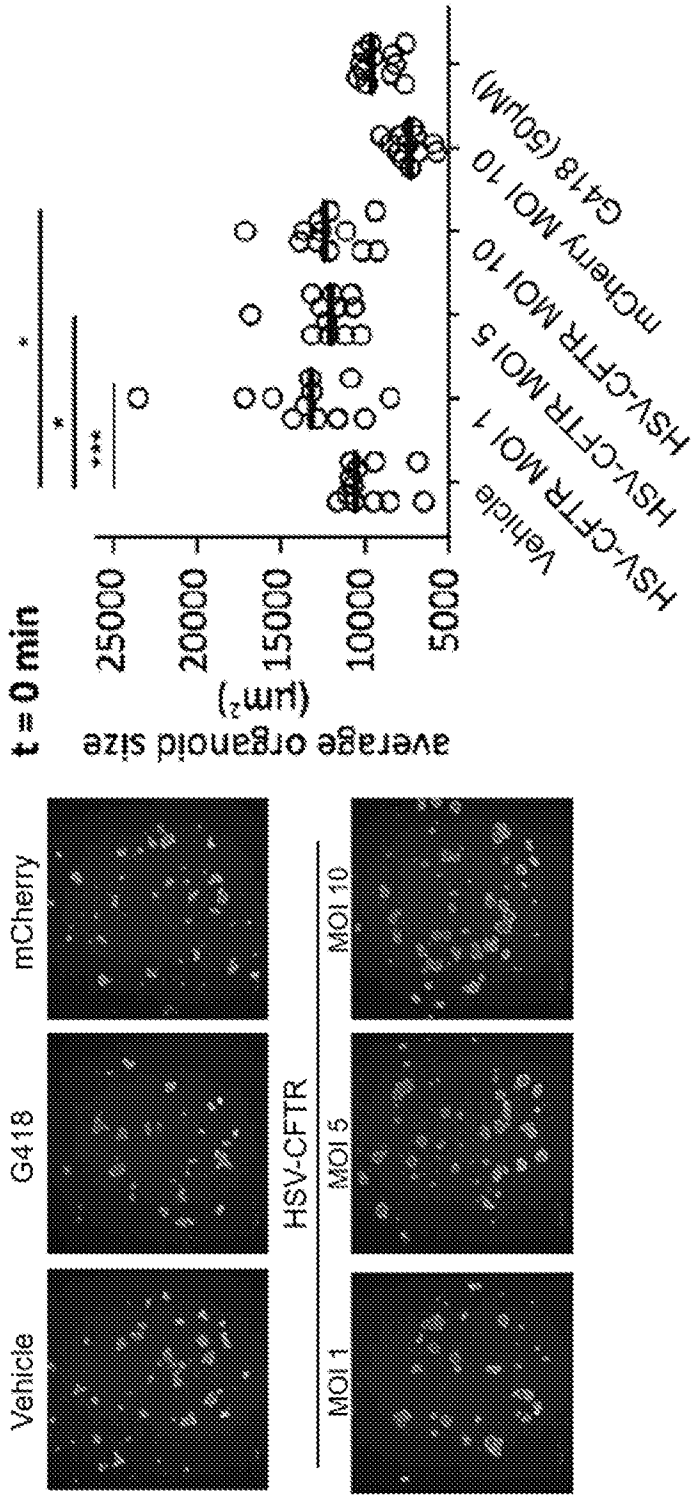

t = 0 min
average organoid size (μm²)
25000
20000
15000
10000
5000
Vehicle
HSV-CFTR MOI 1
HSV-CFTR MOI 5
HSV-CFTR MOI 10
mCherry MOI 10
G418 (50μM)
*
*
***
Vehicle
G418
mCherry
HSV-CFTR
MOI 1
MOI 5
MOI 10

FIG. 8B

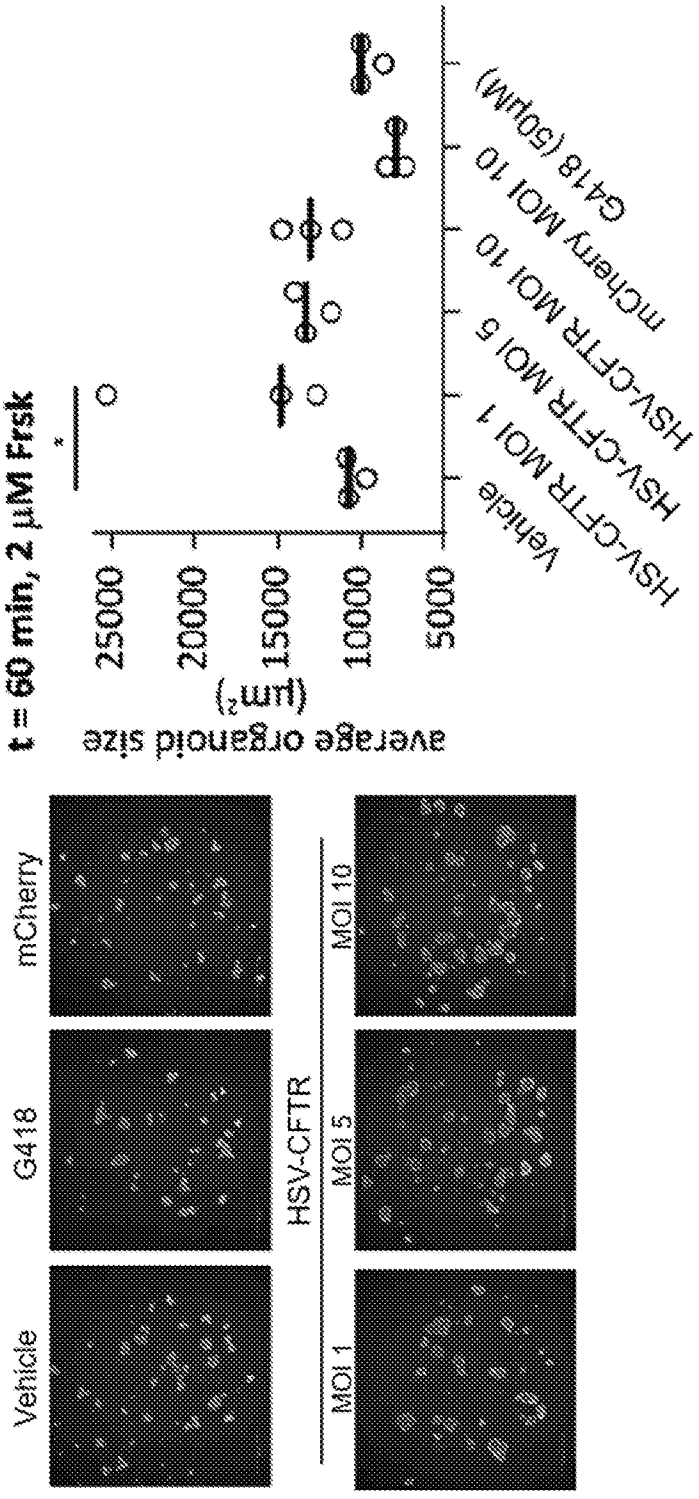

t = 60 min, 2 µM Frsk
average organoid size (µm²)
25000
20000
15000
10000
5000
Vehicle
HSV-CFTR MOI 1
HSV-CFTR MOI 5
HSV-CFTR MOI 10
mCherry MOI 10
G418 (60µM)
Vehicle
G418
mCherry
HSV-CFTR
MOI 1
MOI 5
MOI 10

FIG. 9A

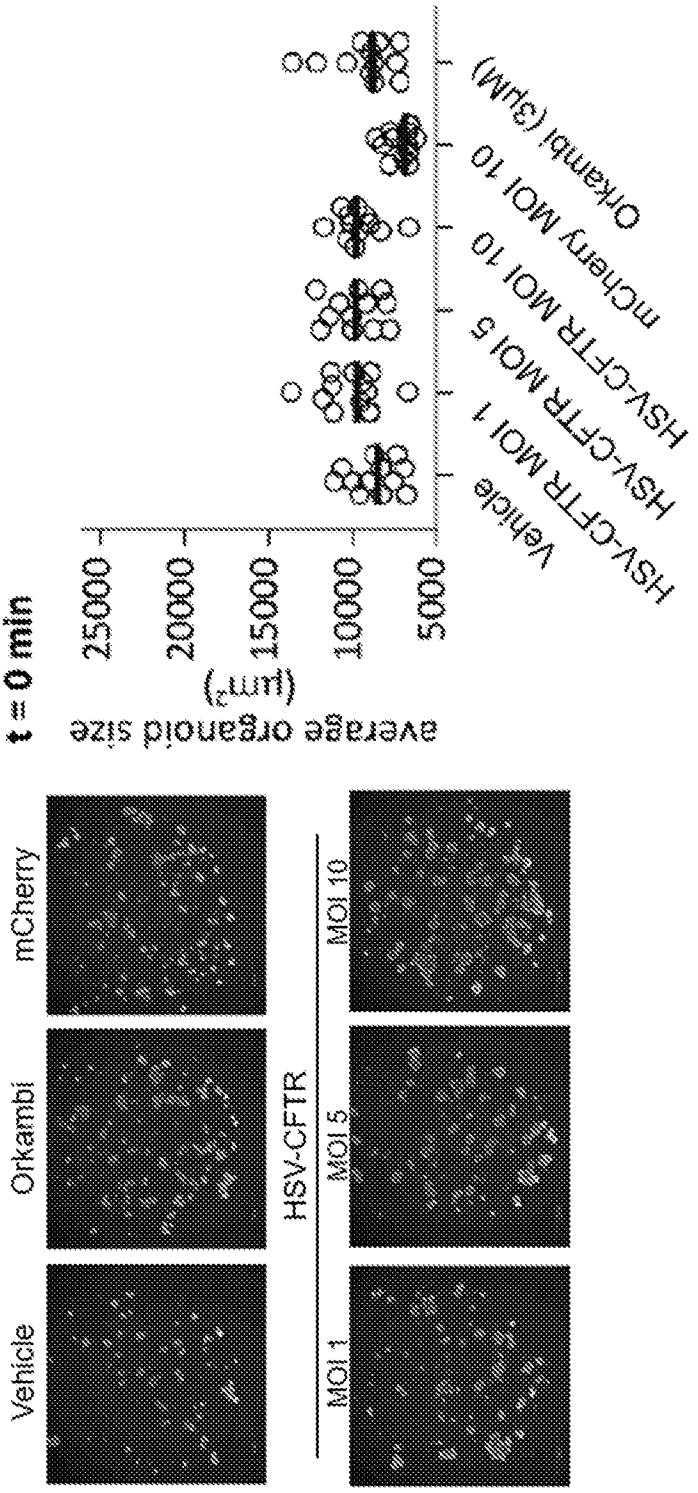

t = 0 min
Vehicle
Orkambi
mCherry
HSV-CFTR
MOI 1
MOI 5
MOI 10
average organoid size (μm²)
25000
20000
15000
10000
5000
Vehicle
HSV-CFTR MOI 1
HSV-CFTR MOI 5
HSV-CFTR MOI 10
mCherry MOI 10
Orkambi (3μM)

FIG. 9B

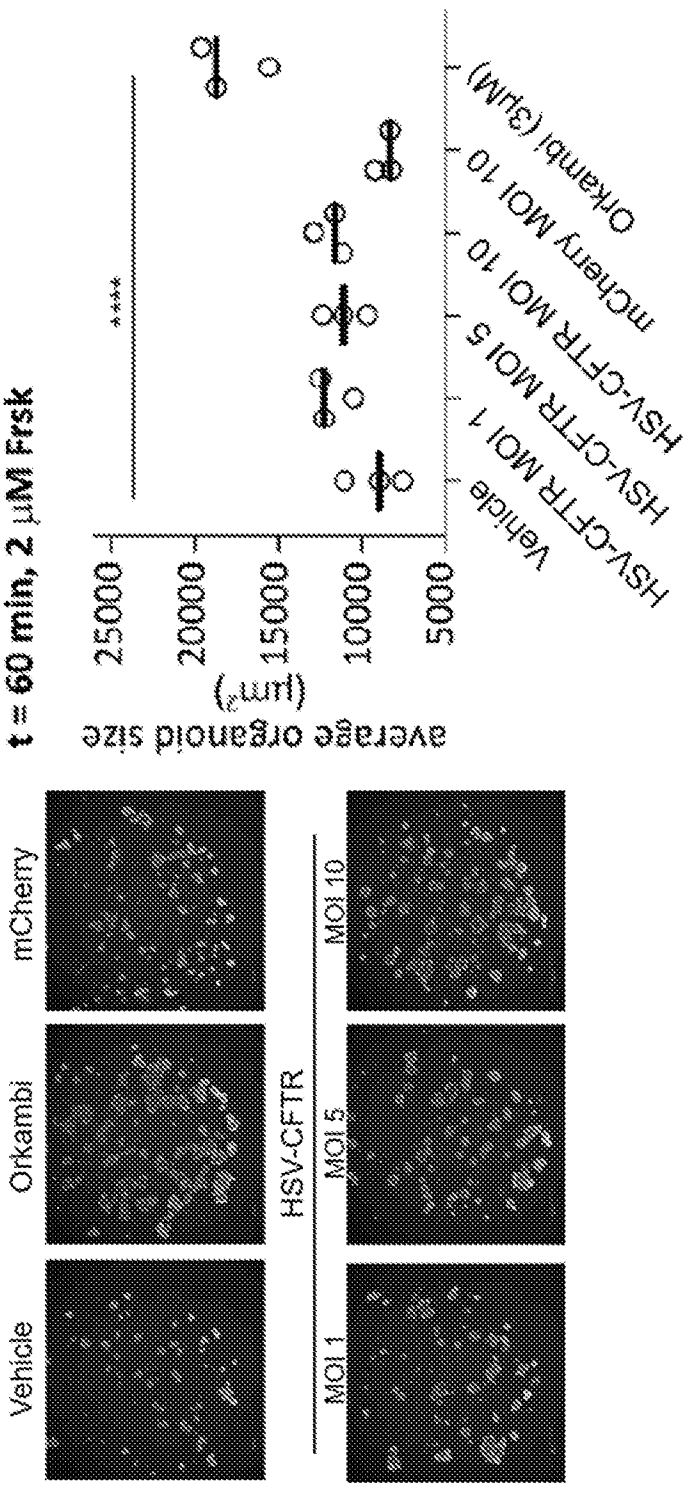

t = 60 min, 2 μM Frsk
average organoid size (μm³)
25000
20000
15000
10000
5000
****
Vehicle
HSV-CFTR MOI 1
HSV-CFTR MOI 5
HSV-CFTR MOI 10
mCherry MOI 10
Orkambi (3μM)
Vehicle
Orkambi
mCherry
HSV-CFTR
MOI 1
MOI 5
MOI 10

FIG. 10A

mCherry Transcripts

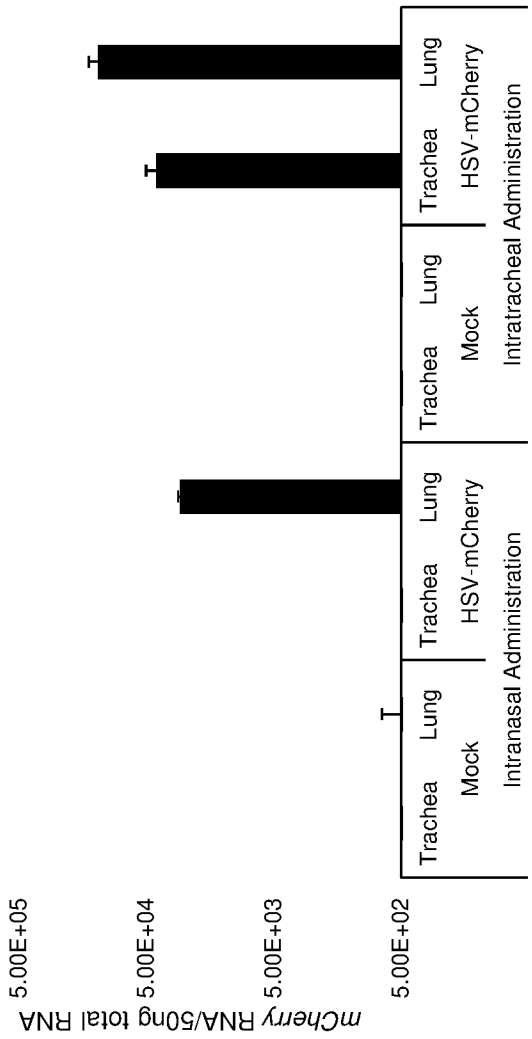


FIG. 10B

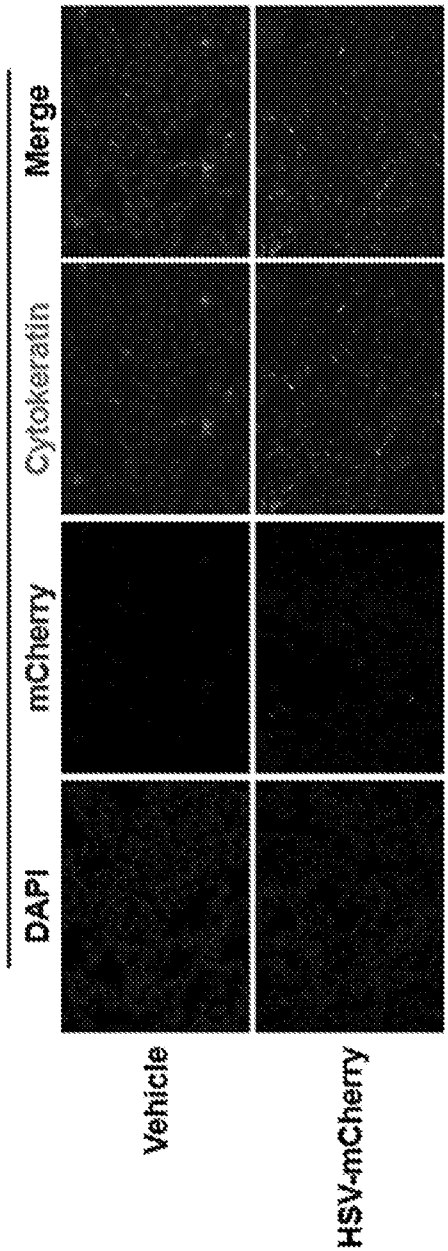
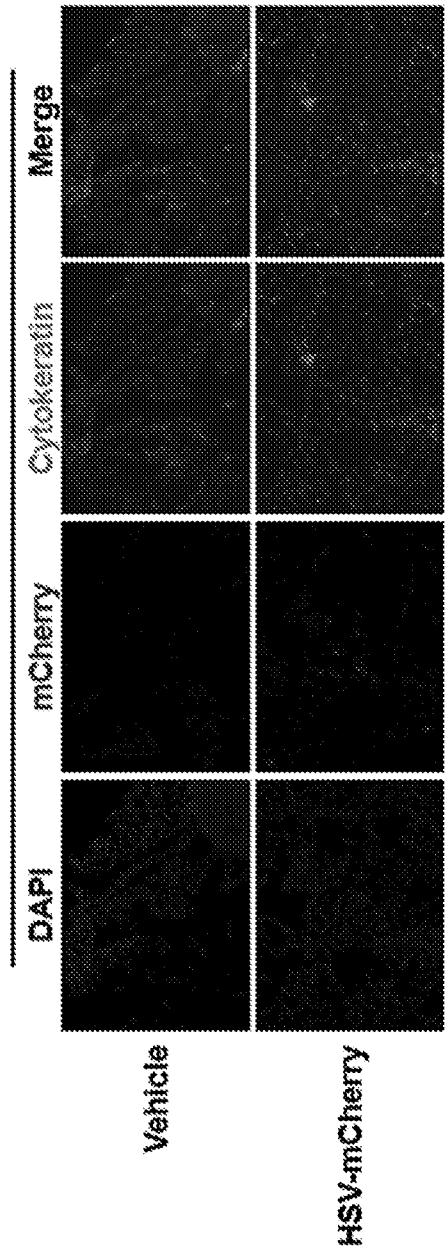


FIG. 10C



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/017191

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47 C12N15/86
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/009952 A1 (UNIV PITTSBURGH [US]; GLORIOSO III JOSEPH C [US] ET AL.) 22 January 2015 (2015-01-22) cited in the application	1-24, 31-50, 55-61,65
Y	page 2, paragraph 0006 page 17, paragraph 0058 page 24, paragraph 0076; claims; examples	1-72
X	WO 98/27216 A1 (UNIV LEEDS [GB]; MARKHAM ALEXANDER FRED [GB]; MEREDITH DAVID MARK [GB]) 25 June 1998 (1998-06-25) page 6, line 5 - page 7, line 18; claims	1-8, 31-38, 43-50
Y	WO 2017/176336 A1 (KRYSTAL BIOTECH LLC [US]) 12 October 2017 (2017-10-12) cited in the application claims; examples	1-72

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
30 April 2020	13/05/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sommer, Birgit

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZIELENSKI J ET AL: "Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene", GENOMICS, ACADEMIC PRESS, SAN DIEGO, US, vol. 10, no. 1, 1 May 1991 (1991-05-01), pages 214-228, XP024797906, ISSN: 0888-7543, DOI: 10.1016/0888-7543(91)90503-7 [retrieved on 1991-05-01] abstract; sequence & DATABASE UniProt [Online] 1 January 1990 (1990-01-01), "RecName: Full=Cystic fibrosis transmembrane conductance regulator; Short=CFTR; AltName: Full=ATP-binding cassette sub-family C member 7; AltName: Full=Channel conductance-controlling ATPase; EC=5.6.1.6 {ECO:0000269¦PubMed:11524016, ECO:0000269¦PubMed:15284228, ECO:0000269¦PubMed:26627831, ECO:000026", retrieved from EBI accession no. UNIPROT:P13569 Database accession no. P13569 sequence -----	1-72
A	COONEY A ET AL: "Cystic Fibrosis Gene Therapy: Looking Back, Looking Forward", GENES, vol. 9, no. 11, 7 November 2018 (2018-11-07), page 538, XP055690547, DOI: 10.3390/genes9110538 items 2 and 3; -----	1-72

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/017191

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		SG 11201808314Q A	30-10-2018
		US 2017290866 A1	12-10-2017
		US 2018169160 A1	21-06-2018
		US 2019160122 A1	30-05-2019
		US 2020101123 A1	02-04-2020
		WO 2017176336 A1	12-10-2017