

COMPOSITIONS AND METHODS FOR TREATING OR PREVENTING HEREDITARY ANGIOEDEMA

Sequence Listing

5 The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on August 11, 2021, is named 51139-026WO2_Sequence_Listing_8_11_21_ST25.txt and is 6,646 bytes in size.

Field of the Invention

10 The disclosure relates to methods for treating hereditary angioedema by way of modulating gene expression, as well as compositions that may be used in such methods.

Background

Hereditary angioedema (HAE) is a disorder that results in recurrent attacks of severe swelling
15 in various body parts, such as the arms, legs, face, intestinal tract, and airway. There is currently no cure for HAE, and long-term, effective treatment options are limited. For patients afflicted with HAE, the disease can have a devastating impact on their lifestyle, as recurrent attacks can happen one or more times per week, with attacks lasting up to three or four days. There remains a need for therapeutic modalities that target underlying causes of HAE to achieve effective amelioration of
20 symptoms and disease remission.

Summary of the Invention

The present disclosure relates to compositions and methods for the treatment of hereditary angioedema (HAE). The present disclosure provides compositions and methods for treating or
25 prevent HAE by administering a viral vector or pluripotent cells modified to secrete therapeutic levels of C1-esterase inhibitor (C1-INH) protein.

In one aspect, the invention features a method of treating HAE in a patient in need thereof by administering to the patient a population of pluripotent cells including a transgene that encodes a C1-INH protein.

30 In another aspect, the invention features a method of inducing sustained remission of HAE in a patient in need thereof by administering to the patient a population of pluripotent cells including a transgene that encodes a C1-INH protein.

In another aspect, the invention features a method of preventing angioedema attacks in a patient diagnosed as having HAE by administering to the patient a population of pluripotent cells
35 including a transgene that encodes a C1-INH protein.

In another aspect, the invention features a method of reducing the risk of recurrent angioedema attacks in a patient diagnosed as having HAE by administering to the patient a population of pluripotent cells including a transgene that encodes a C1-INH protein. In some embodiments, the angioedema attacks occur in the patient's skin, mucosa, gastrointestinal tract,
40 and/or genitourinary region.

In another aspect, the invention features a method of reducing the risk of developing laryngeal angioedema attacks in a patient diagnosed as having HAE by administering to the patient a population of pluripotent cells including a transgene that encodes a C1-INH protein.

In some embodiments of any of the above aspects, the C1-INH transgene is a codon
5 optimized transgene.

In some embodiments of any of the above aspects, the pluripotent cells are hematopoietic stem cells (HSCs) or hematopoietic progenitor cells (HPCs).

In some embodiments, the pluripotent cells are embryonic stem cells.

In some embodiments, the pluripotent cells are induced pluripotent stem cells.

10 In some embodiments, the pluripotent cells are CD34+ cells (e.g., myeloid progenitor cells)

In some embodiments, the population of pluripotent cells is administered systemically (e.g., via intravenous injection) to the patient.

In some embodiments, the pluripotent cells are autologous with respect to the patient.

In some embodiments, the pluripotent cells are allogeneic with respect to the patient.

15 In some embodiments, the pluripotent cells are HLA-matched to the patient.

In some embodiments, the cells are transduced ex vivo to express C1-INH.

In some embodiments, the cells are transduced with a viral vector selected from the group consisting of a Retroviridae family virus, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, and a poxvirus.

20 In some embodiments, the viral vector is a Retroviridae family viral vector.

In some embodiments, the Retroviridae family viral vector is a lentiviral vector.

In some embodiments, the Retroviridae family viral vector is an alpharetroviral vector or a gammaretroviral vector.

25 In some embodiments, the Retroviridae family viral vector includes a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

In some embodiments, the viral vector is a pseudotyped viral vector.

30 In some embodiments, the pseudotyped viral vector selected from the group consisting of a pseudotyped adenovirus, a pseudotyped parvovirus, a pseudotyped coronavirus, a pseudotyped rhabdovirus, a pseudotyped paramyxovirus, a pseudotyped picornavirus, a pseudotyped alphavirus, a pseudotyped herpes virus, a pseudotyped poxvirus, and a pseudotyped Retroviridae family virus.

In some embodiments, the pseudotyped viral vector is a lentiviral vector.

35 In some embodiments, the pseudotyped viral vector includes one or more envelope proteins from a virus selected from vesicular stomatitis virus (VSV), RD114 virus, murine leukemia virus (MLV), feline leukemia virus (FeLV), Venezuelan equine encephalitis virus (VEE), human foamy virus (HFV), walleye dermal sarcoma virus (WDSV), Semliki Forest virus (SFV), Rabies virus, avian leukosis virus (ALV), bovine immunodeficiency virus (BIV), bovine leukemia virus (BLV), Epstein-Barr virus (EBV), Caprine arthritis encephalitis virus (CAEV), Sin Nombre virus (SNV), Cherry Twisted Leaf virus (ChTLV), Simian T-cell leukemia virus (STLV), Mason-Pfizer monkey virus (MPMV), squirrel
40 monkey retrovirus (SMRV), Rous-associated virus (RAV), Fujinami sarcoma virus (FuSV), avian

carcinoma virus (MH2), avian encephalomyelitis virus (AEV), Alfa mosaic virus (AMV), avian sarcoma virus CT10, and equine infectious anemia virus (EIAV).

In some embodiments, the pseudotyped viral vector includes a VSV-G envelope protein.

In some embodiments, the pluripotent cells are transfected ex vivo to express C1-INH.

5 In some embodiments, the pluripotent cells are transfected using a cationic polymer, diethylaminoethyl dextran, polyethylenimine, a cationic lipid, a liposome, calcium phosphate, an activated dendrimer, and/or a magnetic bead.

In some embodiments, the pluripotent cells are transfected by way of electroporation, Nucleofection, squeeze-poration, sonoporation, optical transfection, Magnetofection, and/or
10 impalefection.

In some embodiments, the pluripotent cells are obtained by delivering to the cells a nuclease that catalyzes a single-strand break or a double-strand break at a target position within the genome of the cell, optionally wherein the target position is near or within a gene encoding an endogenous C1-INH protein.

15 In some embodiments, the nuclease is delivered to the cells in combination with a guide RNA (gRNA) that hybridizes to the target position within the genome of the cell.

In some embodiments, the nuclease is a clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein.

In some embodiments, the CRISPR-associated protein is CRISPR-associated protein 9
20 (Cas9) or CRISPR-associated protein 12a (Cas12a).

In some embodiments, the nuclease is a transcription activator-like effector nuclease, a meganuclease, or a zinc finger nuclease.

In some embodiments, the cells are additionally contacted with a template nucleic acid encoding C1-INH while the cells are contacted with the nuclease.

25 In some embodiments, the template nucleic acid molecule encoding C1-INH includes a 5' homology arm and a 3' homology arm having nucleic acid sequences that are sufficiently similar to the nucleic acid sequences located 5' to the target position and 3' to the target position, respectively, to promote homologous recombination.

In some embodiments, the nuclease, gRNA, and/or template nucleic acid are delivered to the
30 cells by contacting the cells with a viral vector that encodes the nuclease, gRNA, and/or template nucleic acid.

In some embodiments, the viral vector that encodes the nuclease, gRNA, and/or template nucleic acid is an AAV, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, a poxvirus, or a Retroviridae family virus.

35 In some embodiments, the viral vector that encodes the nuclease, gRNA, and/or template nucleic acid is a Retroviridae family virus.

In some embodiments, the Retroviridae family virus is a lentiviral vector, alpharetroviral vector, or gammaretroviral vector.

In some embodiments, the Retroviridae family virus that encodes the nuclease, gRNA, and/or
40 template nucleic acid includes a central polypurine tract, a woodchuck hepatitis virus post-

transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

In some embodiments, the viral vector that encodes the nuclease, gRNA, and/or template nucleic acid is an integration-deficient lentiviral vector.

5 In some embodiments, the viral vector that encodes the nuclease, gRNA, and/or template nucleic acid is an AAV selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, and AAVrh74.

In some embodiments, prior to administering the population of pluripotent cells to the patient, a population of precursor cells is isolated from the patient or a donor, and wherein the precursor cells are expanded ex vivo to yield the population of cells being administered to the patient.

10 In some embodiments, the precursor cells are CD34+ HSCs, and wherein the precursor cells are expanded without substantial loss of HSC functional potential.

In some embodiments, prior to isolation of the precursor cells from the patient or donor, the patient or donor is administered one or more pluripotent cell mobilization agents.

15 In some embodiments, prior to administering the population of pluripotent cells to the patient, a population of endogenous pluripotent cells is ablated in the patient by administration of one or more conditioning agents to the patient.

In some embodiments, method includes ablating a population of endogenous pluripotent cells in the patient by administering to the patient one or more conditioning agents prior to administering the population of pluripotent cells to the patient.

20 In some embodiments, the one or more conditioning agents are non-myeloablative conditioning agents.

In some embodiments, the one or more conditioning agents deplete a population of CD34+ cells in the patient.

25 In some embodiments, the depleted CD34+ cells are myeloid progenitor cells.

In some embodiments, the one or more conditioning agents include an antibody or antigen-binding fragment thereof.

In some embodiments, the antibody or antigen-binding fragment thereof binds to CD117, HLA-DR, CD34, CD90, CD45, or CD133.

30 In some embodiments, the antibody or antigen-binding fragment thereof binds to CD117.

In some embodiments, the antibody or antigen-binding fragment thereof is conjugated to a cytotoxin.

In some embodiments, upon administration of the population of pluripotent cells to the patient, the administered cells, or progeny thereof, differentiate into one or more cell types selected from megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeloblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T-lymphocytes, and B-lymphocytes.

In some embodiments, the transgene is operably linked to a ubiquitous promoter. In some embodiments, the transgene is operably linked to a tissue-specific promoter. In some embodiments, the transgene is operably linked to a myeloid cell-specific promoter.

In some embodiments, the transgene is operably linked to a CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, elongation factor 1 α (EF1 α) promoter, EF1 α short form (EFS) promoter, phosphoglycerate kinase (PGK) promoter, α -globin promoter, β -globin promoter, DC172 promoter, human serum albumin promoter, alpha1 antitrypsin promoter, thyroxine binding globulin promoter, or C1-INH promoter.

In some embodiments, the transgene is operably linked to an enhancer.

In some embodiments, the enhancer includes a β -globin locus control region (β LCR).

In some embodiments, the transgene is operably linked to a miRNA targeting sequence

In some embodiments, the miRNA targeting sequence has complementarity to a miRNA that is endogenously expressed in a tissue in which expression of C1-INH is undesirable.

In some embodiments, the patient is a mammal and the cells are mammalian cells. In some embodiments, the mammal is a human and the cells are human cells.

In some embodiments, the patient has a loss-of-function mutation in an endogenous gene encoding C1-INH. For example, the mutation may be a deletion or substitution of an amino acid located within the reactive center loop (RCL) of C1-INH. The mutation may be a deletion or substitution of K251. The mutation may be selected from the group consisting of A436T, R444H, R444C, R444S, V432E, A443V, Y199TER, I462S, and R378C.

In some embodiments, the patient has a mutation in an endogenous gene encoding C1-INH that causes (i) a deletion or (ii) expression of a truncated transcript.

In some embodiments, the patient has a mutation in a gene encoding coagulation factor XII (F12).

In some embodiments, the mutation is heterozygous. In some embodiments, the mutation is homozygous.

In some embodiments, the patient has previously been treated with one or more immunosuppressive agents, biologic agents, and/or corticosteroids. In some embodiments, the patient has not responded to treatment with the one or more immunosuppressive agents, biologic agents, and/or corticosteroids.

In some embodiments, the patient has previously been treated with one or more therapeutic agents selected from the group consisting of C1-esterase inhibitor (e.g., BERINERT® or RUCONEST®), icatibant (e.g., icatibant injection, e.g., FIRAZYR®), and ecallantide (e.g., KALBITOR®). In some embodiments, the patient has not responded to treatment with the one or more therapeutic agents.

In some embodiments, the patient has previously been treated with one or more prophylactic agents selected from the group consisting of Cinryze, Haegarda, Takhzyro, and an androgen. In some embodiments, the patient has not responded to treatment with the one or more prophylactic agents.

In some embodiments, the patient is less than 12 years old (e.g., less than 6 years old). In some embodiments, the patient is more than 6 years old (e.g., more than 12 years old).

In some embodiments, prior to administering the population of pluripotent cells to the patient, the patient exhibits angioedema attacks with a frequency of from one to ten times per month.

In some embodiments, prior to administering the population of pluripotent cells to the patient, the patient exhibits angioedema attacks with a frequency of one or two times per week.

In some embodiments, after administering the population of pluripotent cells to the patient, the patient exhibits sustained disease remission.

5 In some embodiments, after administering the population of pluripotent cells to the patient, the patient does not exhibit an angioedema attack for a period of from about two months to about one year.

10 In some embodiments, after administering the population of pluripotent cells to the patient, the patient exhibits a serum concentration of C1-INH protein of at least about 7 mg/dl (e.g., from about from about 15 mg/dl to about 35 mg/dl).

15 In some embodiments, after administering the population of pluripotent cells to the patient, the patient exhibits a serum concentration of C1-INH protein that is from about 40% to about 60% of a serum concentration of C1-INH protein exhibited by a subject that does not have HAE, optionally wherein the subject (i) is the same gender as the patient and/or (ii) has the same body mass index as the patient.

In some embodiments, administration of the population of pluripotent cells to the patient reduces the patient's risk of suffocation due to laryngeal angioedema attacks.

In another aspect, the invention features a method of treating HAE in a patient in need thereof by administering to the patient a lentiviral vector including a transgene that encodes a C1-INH protein.

20 In another aspect, the invention features a method of inducing sustained remission of HAE in a patient in need thereof by administering to the patient a lentiviral vector including a transgene that encodes a C1-INH protein.

25 In another aspect, the invention features a method of preventing angioedema attacks in a patient diagnosed as having HAE by administering to the patient a lentiviral vector including a transgene that encodes a C1-INH protein.

In another aspect, the invention features a method of reducing the risk of recurrent angioedema attacks in a patient diagnosed as having HAE by administering to the patient a lentiviral vector including a transgene that encodes a C1-INH protein.

30 In some embodiments, the angioedema attacks occur in the patient's skin, mucosa, gastrointestinal tract, and/or genitourinary region.

In another aspect, the invention features a method of reducing the risk of developing laryngeal angioedema attacks in a patient diagnosed as having HAE by administering to the patient a lentiviral vector including a transgene that encodes a C1-INH protein.

35 In some embodiments, the lentiviral vector is administered systemically (e.g., via intravenous injection) to the patient.

In some embodiments, the lentiviral vector includes a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

40 In some embodiments, the lentiviral vector is pseudotyped. The lentiviral vector may include one or more envelope proteins from a virus selected from VSV, RD114 virus, MLV, FeLV, VEE, HFV,

WDSV, SFV, Rabies virus, ALV, BIV, BLV, EBV, CAEV, SNV, ChTLV, STLV, MPMV, SMRV, RAV, FuSV, MH2, AEV, AMV, avian sarcoma virus CT10, and EIAV.

In some embodiments, the lentiviral vector includes a VSV-G envelope protein.

In some embodiments, the transgene is operably linked to a ubiquitous promoter. In some
5 embodiments, the transgene is operably linked to a tissue-specific promoter. In some embodiments, the transgene is operably linked to a hepatocyte-specific promoter.

In some embodiments, the transgene is operably linked to a transthyretin promoter, CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, EF1 α promoter, EFS promoter, PGK promoter, α -globin promoter, β -globin promoter, DC172 promoter, human serum albumin
10 promoter, alpha1 antitrypsin promoter, thyroxine binding globulin promoter, or C1-INH promoter.

In some embodiments, the transgene is operably linked to an enhancer. The enhancer may include a β LCR.

In some embodiments, the transgene is operably linked to a miRNA targeting sequence

In some embodiments, the miRNA targeting sequence has complementarity to a miRNA that
15 is endogenously expressed in a tissue in which expression of C1-INH is undesirable.

In some embodiments, the patient is a mammal (e.g., a human).

In some embodiments, the patient has a loss-of-function mutation in an endogenous gene encoding C1-INH. The mutation may be a deletion or a substitution of an amino acid located within the RCL of C1-INH. The mutation may be deletion or substitution of K251. The mutation may be
20 selected from the group consisting of A436T, R444H, R444C, R444S, V432E, A443V, Y199TER, I462S, and R378C.

In some embodiments, the patient has a mutation in an endogenous gene encoding C1-INH that causes (i) a deletion or (ii) expression of a truncated transcript.

In some embodiments, the patient has a mutation in a gene encoding F12.

In some embodiments, the mutation is heterozygous. In some embodiments, the mutation is
25 homozygous.

In some embodiments, the patient has previously been treated with one or more immunosuppressive agents, biologic agents, and/or corticosteroids. The patient may not have responded to treatment with the one or more immunosuppressive agents, biologic agents, and/or
30 corticosteroids.

In some embodiments, the patient has previously been treated with one or more therapeutic agents selected from the group consisting of C1-esterase inhibitor (e.g., BERINERT® or RUCONEST®), icatibant (e.g., e.g., icatibant injection, e.g., FIRAZYR®), and ecallantide (e.g., KALBITOR®). The patient may not have responded to treatment with the one or more therapeutic
35 agents.

In some embodiments, the patient has previously been treated with one or more prophylactic agents selected from the group consisting of Cinryze, Haegarda, Takhzyro, and an androgen. The patient may not have responded to treatment with the one or more prophylactic agents.

In some embodiments, the patient is less than 12 years old (e.g., less than 6 years old). In
40 some embodiments, the patient is more than 6 years old (e.g., more than 12 years old).

In some embodiments, prior to administering the lentiviral vector to the patient, the patient exhibits angioedema attacks with a frequency of from one to ten times per month.

In some embodiments, prior to administering the lentiviral vector to the patient, the patient exhibits angioedema attacks with a frequency of one or two times per week.

5 In some embodiments, after administering the lentiviral vector to the patient, the patient exhibits sustained disease remission.

In some embodiments, after administering the lentiviral vector to the patient, the patient does not exhibit an angioedema attack for a period of from about two months to about one year.

10 In some embodiments, after administering the lentiviral vector to the patient, the patient exhibits a serum concentration of C1-INH protein of at least about 7 mg/dl (e.g., from about 15 mg/dl to about 35 mg/dl).

In some embodiments, after administering the lentiviral vector to the patient, the patient exhibits a serum concentration of C1-INH protein that is from about 40% to about 60% of a serum concentration of C1-INH protein exhibited by a subject that does not have HAE, optionally wherein the
15 subject (i) is the same gender as the patient and/or (ii) has the same body mass index as the patient.

In some embodiments, administration of the lentiviral vector to the patient reduces the patient's risk of suffocation due to laryngeal angioedema attacks.

In another aspect, the invention features a pharmaceutical composition that includes (i) a population of pluripotent cells including a transgene that encodes a C1-INH protein and (ii) one or
20 more carriers, diluents, and/or excipients.

The cells may be human cells. The cells may be HSCs or HPCs. The cells may be embryonic stem cells. The cells may be induced pluripotent stem cells. The cells may be CD34+ cells (e.g., myeloid progenitor cells).

25 In some embodiments, the composition is formulated for administration (e.g., via intravenous injection) to a human patient.

In some embodiments, the cells are autologous with respect to the patient. In some embodiments, the cells are allogeneic with respect to the patient. The cells may be HLA-matched to the patient.

30 In some embodiments, the transgene is operably linked to a ubiquitous promoter. In some embodiments, the transgene is operably linked to a tissue-specific promoter. In some embodiments, the transgene is operably linked to a myeloid cell-specific promoter.

In some embodiments, the transgene is operably linked to a CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, EF1 α promoter, EFS promoter, PGK promoter, α -globin promoter, β -globin promoter, DC172 promoter, human serum albumin promoter, alpha1
35 antitrypsin promoter, thyroxine binding globulin promoter, or C1-INH promoter.

In some embodiments, the transgene is operably linked to an enhancer. The enhancer may include a β LCR.

40 In some embodiments, the transgene is operably linked to a miRNA targeting sequence. The miRNA targeting sequence may have complementarity to a miRNA that is endogenously expressed in a tissue in which expression of C1-INH is undesirable.

In another aspect, the invention features a pharmaceutical composition including (i) a lentiviral vector including a transgene that encodes a C1-INH protein and (ii) one or more carriers, diluents, and/or excipients.

In some embodiments, the lentiviral vector includes a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

In some embodiments, the lentiviral vector is pseudotyped. The lentiviral vector may include one or more envelope proteins from a virus selected from VSV, RD114 virus, MLV, FeLV, VEE, HFV, WDSV, SFV, Rabies virus, ALV, BIV, BLV, EBV, CAEV, SNV, ChTLV, STLV, MPMV, SMRV, RAV, FuSV, MH2, AEV, AMV, avian sarcoma virus CT10, and EIAV. The lentiviral vector may include a VSV-G envelope protein.

In some embodiments, the composition is formulated for administration (e.g., via intravenous injection) to a human patient.

In some embodiments, the transgene is operably linked to a ubiquitous promoter. In some embodiments, the transgene is operably linked to a tissue-specific promoter. In some embodiments, the transgene is operably linked to a hepatocyte-specific promoter. The transgene may be operably linked to a transthyretin promoter, CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, EF1 α promoter, EFS promoter, PGK promoter, α -globin promoter, β -globin promoter, DC172 promoter, human serum albumin promoter, alpha1 antitrypsin promoter, thyroxine binding globulin promoter, or C1-INH promoter.

In some embodiments, the transgene is operably linked to an enhancer. The transgene may include a β LCR.

In some embodiments, the transgene is operably linked to a miRNA targeting sequence. The miRNA targeting sequence may have complementarity to a miRNA that is endogenously expressed in a tissue in which expression of C1-INH is undesirable.

In another aspect, the invention features a kit including a pharmaceutical composition as described herein. The kit may further include a package insert instructing a user of the kit to administer the pharmaceutical composition to a human patient having HAE. The package insert may instruct a user of the kit to perform a method as described herein.

Definitions

As used herein, the terms "ablate," "ablating," "ablation," "condition," "conditioning," and the like refer to the depletion of one or more cells in a population of cells in vivo or ex vivo. In some embodiments of the present disclosure, it may be desirable to ablate endogenous cells within a patient (e.g., a patient undergoing treatment for a disease described herein) before administering a therapeutic composition, such as a therapeutic population of cells, to the patient. This can be beneficial, for example, in order to provide newly-administered cells with an environment within which the cells may engraft. Ablation of a population of endogenous cells can be performed in a manner that selectively targets a specific cell type, for example, using antibodies or antibody-drug conjugates that bind to an antigen expressed on the target cell and subsequently engender the killing of the

target cell. Additionally, or alternatively, ablation may be performed in a non-specific manner using cytotoxins that do not localize to a particular cell type but are instead capable of exerting their cytotoxic effects on a variety of different cells. Examples of ablation include depletion of at least 5% of cells (e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more) in a population of cells in vivo or in vitro. Quantifying cell counts within a sample of cells can be performed using a variety of cell-counting techniques, such as through the use of a counting chamber, a Coulter counter, flow cytometry, or other cell-counting methods known in the art.

Exemplary agents that can be used to "ablate" a population of cells in a patient (i.e., to "condition") a patient for treatment) in accordance with the compositions and methods of the disclosure include alkylating agents, such as nitrogen mustards (e.g., bendamustine, chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, or melphalan), nitrosoureas (e.g., carmustine, lomustine, or streptozocin), alkyl sulfonates (e.g., busulfan), triazines (e.g., dacarbazine or temozolomide), or ethylenimines (e.g., altretamine or thiotepa). In some embodiments, the one or more conditioning agents are non-myeloablative conditioning agents that selectively target and ablate a specific population of endogenous pluripotent cells, such as a population of endogenous CD34+ HSCs or HPCs. For example, the one or more conditioning agents may include cytarabine, antithymocyte globulin, fludarabine, or idarubicin.

As used herein, the term "about" refers to a quantity that varies by as much as 30% (e.g., 25%, 20%, 25%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%) relative to a reference quantity.

As used herein in the context of a protein of interest, the term "activity" refers to the biological functionality that is associated with a wild-type form of the protein. For example, in the context of an enzyme, the term "activity" refers to the ability of the protein to effectuate substrate turnover in a manner that yields the product of a corresponding chemical reaction. Activity levels of enzymes can be detected and quantitated, for example, using substrate turnover assays known in the art. As another example, in the context of a membrane-bound receptor, the term "activity" may refer to signal transduction initiated by the receptor, e.g., upon binding to its cognate ligand. Activity levels of receptors involved in signal transduction pathways can be detected and quantitated, for example, by observing an increase in the outcome of receptor signaling, such as an increase in the transcription of one or more genes (which may be detected, e.g., using polymerase chain reaction techniques known in the art).

As used herein, the terms "administering," "administration," and the like refer to directly giving a patient a therapeutic agent (e.g., a population of cells, such as a population of pluripotent cells (e.g., embryonic stem cells, induced pluripotent stem cells, or CD34+ cells)) by any effective route. Exemplary routes of administration are described herein and include systemic administration routes, such as intravenous injection, among others.

As used herein, the term "allogeneic" refers to cells, tissues, nucleic acid molecules, or other substances obtained or derived from a different subject of the same species. For example, in the context of a population of cells (e.g., a population of pluripotent cells) expressing one or more proteins described herein, allogeneic cells include those that are (i) obtained from a subject that is not undergoing therapy and are then (ii) transduced or transfected with a vector that directs the

expression of one or more desired proteins. The phrase "directs expression" refers to the inclusion of one or more polynucleotides encoding the one or more proteins to be expressed. The polynucleotide may contain additional sequence motifs that enhances expression of the protein of interest.

As used herein, the term "autologous" refers to cells, tissues, nucleic acid molecules, or other substances obtained or derived from an individual's own cells, tissues, nucleic acid molecules, or the like. For example, in the context of a population of cells (e.g., a population of pluripotent cells) expressing one or more proteins described herein, autologous cells include those that are obtained from the patient undergoing therapy that are then transduced or transfected with a vector that directs the expression of one or more proteins of interest.

As used herein, the term "cell type" refers to a group of cells sharing a phenotype that is statistically separable based on gene expression data. For example, cells of a common cell type may share similar structural and/or functional characteristics, such as similar gene activation patterns and antigen presentation profiles. Cells of a common cell type may include those that are isolated from a common tissue (e.g., epithelial tissue, neural tissue, connective tissue, or muscle tissue) and/or those that are isolated from a common organ, tissue system, blood vessel, or other structure and/or region in an organism.

As used herein, "codon optimization" refers a process of modifying a nucleic acid sequence in accordance with the principle that the frequency of occurrence of synonymous codons (e.g., codons that code for the same amino acid) in coding DNA is biased in different species. Such codon degeneracy allows an identical polypeptide to be encoded by a variety of nucleotide sequences. Sequences modified in this way are referred to herein as "codon-optimized." This process may be performed on any of the sequences described in this specification to enhance expression or stability. Codon optimization may be performed in a manner such as that described in, e.g., U.S. Patent Nos. 7,561,972, 7,561,973, and 7,888,112, each of which is incorporated herein by reference in its entirety. The sequence surrounding the translational start site can be converted to a consensus Kozak sequence according to known methods. See, e.g., Kozak et al, Nucleic Acids Res.15 (20): 8125-8148, incorporated herein by reference in its entirety. Multiple stop codons can be incorporated.

As used herein, the terms "condition" and "conditioning" refer to processes by which a subject is prepared for receipt of a transplant containing a population of cells (e.g., a population of pluripotent cells, such as CD34+ cells). Such procedures promote the engraftment of a cell transplant, for example, by selectively depleting endogenous cells (e.g., endogenous CD34+ cells, among others) thereby creating a vacancy which is in turn filled by the exogenous cell transplant. According to the methods described herein, a subject may be conditioned for cell transplant procedure by administration to the subject of one or more agents capable of ablating endogenous cells (e.g., CD34+ cells, among others), radiation therapy, or a combination thereof. Conditioning regimens useful in conjunction with the compositions and methods of the disclosure may be myeloablative or non-myeloablative. Other cell-ablating agents and methods well known in the art (e.g., antibodies and antibody-drug conjugates) may also be used.

As used herein, the terms "conservative mutation," "conservative substitution," "conservative amino acid substitution," and the like refer to a substitution of one or more amino acids for one or

more different amino acids that exhibit similar physicochemical properties, such as polarity, electrostatic charge, and steric volume. These properties are summarized for each of the twenty naturally-occurring amino acids in Table 1 below.

5 **Table 1. Representative physicochemical properties of naturally occurring amino acids**

Amino Acid	3 Letter Code	1 Letter Code	Side-chain Polarity	Electrostatic character at physiological pH (7.4)	Steric Volume [†]
Alanine	Ala	A	nonpolar	neutral	small
Arginine	Arg	R	polar	cationic	large
Asparagine	Asn	N	polar	neutral	intermediate
Aspartic acid	Asp	D	polar	anionic	intermediate
Cysteine	Cys	C	nonpolar	neutral	intermediate
Glutamic acid	Glu	E	polar	anionic	intermediate
Glutamine	Gln	Q	polar	neutral	intermediate
Glycine	Gly	G	nonpolar	neutral	small
Histidine	His	H	polar	Both neutral and cationic forms in equilibrium at pH 7.4	large
Isoleucine	Ile	I	nonpolar	neutral	large
Leucine	Leu	L	nonpolar	neutral	large
Lysine	Lys	K	polar	cationic	large
Methionine	Met	M	nonpolar	neutral	large
Phenylalanine	Phe	F	nonpolar	neutral	large
Proline	Pro	P	non-polar	neutral	intermediate
Serine	Ser	S	polar	neutral	small
Threonine	Thr	T	polar	neutral	intermediate
Tryptophan	Trp	W	nonpolar	neutral	bulky
Tyrosine	Tyr	Y	polar	neutral	large
Valine	Val	V	nonpolar	neutral	intermediate

[†]based on volume in Å³: 50-100 is small, 100-150 is intermediate, 150-200 is large, and >200 is bulky

From this table it is appreciated that the conservative amino acid families include (i) G, A, V, L and I; (ii) D and E; (iii) C, S and T; (iv) H, K and R; (v) N and Q; and (vi) F, Y and W. A conservative mutation or substitution is therefore one that substitutes one amino acid for a member of the same amino acid family (e.g., a substitution of Ser for Thr or Lys for Arg).

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As used herein in the context of a gene of interest, the term "disrupt" refers to preventing the formation of a functional gene product. A gene product is considered to be functional according to the present disclosure if it fulfills its normal (wild type) function(s). Disruption of the gene prevents expression of a functional factor (e.g., protein) encoded by the gene and may be achieved, for example, by way of an insertion, deletion, or substitution of one or more bases in a sequence encoded by the gene and/or a promoter and/or an operator that is necessary for expression of the gene in a subject. The disrupted gene may be disrupted by, e.g., removal of at least a portion of the gene from a genome of the subject, alteration of the gene to prevent expression of a functional factor (e.g., protein) encoded by the gene, an interfering RNA, or expression of a dominant negative factor by an exogenous gene. Materials and methods for genetically modifying cells (e.g., pluripotent cells, such as CD34+ cells, hematopoietic stem cells, and myeloid progenitor cells, among others) so as to disrupt the expression of one or more genes are detailed, for example, in US 8,518,701; US 9,499,808; and US 2012/0222143, the disclosures of each of which are incorporated herein by reference in their entirety (in case of conflict, the instant specification is controlling).

As used herein, the terms "embryonic stem cell" and "ES cell" refer to an embryo-derived totipotent or pluripotent stem cell, derived from the inner cell mass of a blastocyst that can be maintained in an in vitro culture under suitable conditions. ES cells are capable of differentiating into cells of any of the three vertebrate germ layers, e.g., the endoderm, the ectoderm, or the mesoderm. ES cells are also characterized by their ability to propagate indefinitely under suitable in vitro culture conditions. ES cells are described, for example, in Thomson et al., Science 282:1145 (1998), the disclosure of which is incorporated herein by reference as it pertains to the structure and functionality of embryonic stem cells.

As used herein, the term "endogenous" describes a molecule (e.g., a polypeptide, nucleic acid, or cofactor) that is found naturally in a particular organism (e.g., a human) or in a particular location within an organism (e.g., an organ, a tissue, or a cell, such as a human cell).

As used herein, the term "exogenous" describes a molecule (e.g., a polypeptide, nucleic acid, or cofactor) that is not found naturally in a particular organism (e.g., a human) or in a particular location within an organism (e.g., an organ, a tissue, or a cell, such as a human cell). Exogenous materials include those that are provided from an external source to an organism or to cultured matter extracted there from.

As used herein, the term "expansion agent" refers to a substance capable of promoting the proliferation of a given cell type ex vivo. Accordingly, a "hematopoietic stem cell expansion agent" or an "HSC expansion agent" refers to a substance capable of promoting the proliferation of a population of hematopoietic stem cells ex vivo. Hematopoietic stem cell expansion agents include those that effectuate the proliferation of a population of hematopoietic stem cells such that the cells retain hematopoietic stem cell functional potential. Exemplary hematopoietic stem cell expansion agents that may be used in conjunction with the compositions and methods of the disclosure include, without limitation, aryl hydrocarbon receptor antagonists, such as those described in US Patent Nos. 8,927,281 and 9,580,426, the disclosures of each of which are incorporated herein by reference in their entirety, and, in particular, compound SR1. Additional hematopoietic stem cell expansion agents

that may be used in conjunction with the compositions and methods of the disclosure include compound UM-171 and other compounds described in US Patent No. 9,409,906, the disclosure of which is incorporated herein by reference in its entirety. Hematopoietic stem cell expansion agents further include structural and/or stereoisomeric variants of compound UM-171, such as the compounds described in US 2017/0037047, the disclosure of which is incorporated herein by reference in its entirety. Additional hematopoietic stem cell expansion agents suitable for use in the instant disclosure include histone deacetylase (HDAC) inhibitors, such as trichostatin A, trapoxin, trapoxin A, chlamydocin, sodium butyrate, dimethyl sulfoxide, suberanilohydroxamic acid, m-carboxycinnamic acid bishydroxamide, HC-toxin, Cyl-2, WF-3161, depudecin, and radicicol, among others described, for example, in WO 2000/023567, the disclosure of which is incorporated herein by reference. Additional hematopoietic stem cell expansion agents include valproic acid, e.g., as described in De Felice et al, *Cancer Res* 65: 1505-13, 2005, hereby incorporated by reference.

As used herein, the term "express" refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein. In the context of a gene that encodes a protein product, the terms "gene expression" and the like are used interchangeably with the terms "protein expression" and the like. Expression of a gene or protein of interest in a subject can manifest, for example, by detecting: an increase in the quantity or concentration of mRNA encoding corresponding protein (as assessed, e.g., using RNA detection procedures described herein or known in the art, such as quantitative polymerase chain reaction (qPCR) and RNA seq techniques), an increase in the quantity or concentration of the corresponding protein (as assessed, e.g., using protein detection methods described herein or known in the art, such as enzyme-linked immunosorbent assays (ELISA), among others), and/or an increase in the activity of the corresponding protein (e.g., in the case of an enzyme, as assessed using an enzymatic activity assay described herein or known in the art) in a sample obtained from the subject. As used herein, a cell is considered to "express" a gene or protein of interest if one or more, or all, of the above events can be detected in the cell or in a medium in which the cell resides. For example, a gene or protein of interest is considered to be "expressed" by a cell or population of cells if one can detect (i) production of a corresponding RNA transcript, such as an mRNA template, by the cell or population of cells (e.g., using RNA detection procedures described herein); (ii) processing of the RNA transcript (e.g., splicing, editing, 5' cap formation, and/or 3' end processing, such as using RNA detection procedures described herein); (iii) translation of the RNA template into a protein product (e.g., using protein detection procedures described herein); and/or (iv) post-translational modification of the protein product (e.g., using protein detection procedures described herein).

As used herein, the term "functional potential" as it pertains to a pluripotent cell, such as a hematopoietic stem cell, refers to the functional properties of stem cells which include: 1) multipotency (which refers to the ability to differentiate into multiple different blood lineages including, but not limited to granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing

megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells); 2) self-renewal (which refers to the ability of stem cells to give rise to daughter cells that have equivalent potential as the mother cell, and further that this ability can repeatedly occur throughout the lifetime of an individual without

5 exhaustion); and 3) the ability of stem cells or progeny thereof to be reintroduced into a transplant recipient whereupon they home to the stem cell niche and re-establish productive and sustained cell growth and differentiation.

As used herein, the terms "hematopoietic stem cells" and "HSCs" refer to immature blood cells having the capacity to self-renew and to differentiate into mature blood cells of diverse lineages including but not limited to granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells). It is known in the art that such cells may or may not include CD34+ cells. CD34+ cells are immature cells that express the

15 CD34 cell surface marker. In humans, CD34+ cells are believed to include a subpopulation of cells with the stem cell properties defined above, whereas in mice, HSCs are CD34-. In addition, HSCs also refer to long term repopulating HSC (LT-HSC) and short-term repopulating HSC (ST-HSC). LT-HSC and ST-HSC are differentiated, based on functional potential and on cell surface marker expression. For example, human HSC can be CD34+, CD38-, CD45RA-, CD90+, CD49F+, and lin- (negative for mature lineage markers including CO2, CD3, CD4, CD7, CD8, CD10, CD11B, CD19, CD20, CD56, CD235A). In mice, bone marrow LT-HSC can be CD34-, SCA-1+, C-kit+, CD135-, Slamf1/CD150+, CD48-, and lin- (negative for mature lineage markers including Ter119, CD11b, Gr1, CD3, CD4, CD8, B220, IL-7ra), whereas ST-HSC can be CD34+, SCA-1+, C-kit+, CD135-, Slamf1/CD150+, and lin- (negative for mature lineage markers including Ter119, CD11b, Gr1, CD3,

25 CD4, CD8, B220, IL-7ra). In addition, ST-HSC are less quiescent (i.e., more active) and more proliferative than L T-HSC under homeostatic conditions. However, LT-HSC have greater self-renewal potential (i.e., they survive throughout adulthood, and can be serially transplanted through successive recipients), whereas ST-HSC have limited self-renewal (i.e., they survive for only a limited period of time, and do not possess serial transplantation potential). Any of these HSCs can be used

30 in any of the methods described herein. Optionally, ST-HSCs are useful because they are highly proliferative and thus, can more quickly give rise to differentiated progeny.

As used herein, an agent that inhibits histone deacetylation refers to a substance or composition (e.g., a small molecule, protein, interfering RNA, messenger RNA, or other natural or synthetic compound, or a composition such as a virus or other material composed of multiple

35 substances) capable of attenuating or preventing the activity of histone deacetylase, more particularly its enzymatic activity either via direct interaction or via indirect means such as by causing a reduction in the quantity of a histone deacetylase produced in a cell or by inhibition of the interaction between a histone deacetylase and an acetylated histone substrate. Inhibiting histone deacetylase enzymatic activity means reducing the ability of a histone deacetylase to catalyze the removal of an acetyl group

40 from a histone residue (e.g., a mono-, di-, or tri-methylated lysine residue; a monomethylated arginine

residue, or a symmetric/asymmetric dimethylated arginine residue, within a histone protein). Preferably, such inhibition is specific, such that the agent that inhibits histone deacetylation reduces the ability of a histone deacetylase to remove an acetyl group from a histone residue at a concentration that is lower than the concentration of the inhibitor that is required to produce another, unrelated biological effect.

As used herein, the terms "histone deacetylase" and "HDAC" refer to any one of a family of enzymes that catalyze the removal of acetyl groups from the ϵ -amino groups of lysine residues at the N-terminus of a histone. Unless otherwise indicated by context, the term "histone" is meant to refer to any histone protein, including H1, H2A, H2B, H3, H4, and H5, from any species. Human HDAC proteins or gene products, include, but are not limited to, HDAC-1, HDAC-2, HDAC-3, HDAC-4, HDAC-5, HDAC-6, HDAC-7, HDAC-8, HDAC-9, HDAC-10, and HDAC-11.

As used herein, the term "HLA-matched" refers to a donor-recipient pair in which none of the HLA antigens are mismatched between the donor and recipient, such as a donor providing a hematopoietic stem cell graft to a recipient in need of hematopoietic stem cell transplant therapy.

HLA-matched (i.e., where all of the 6 alleles are matched) donor-recipient pairs have a decreased risk of graft rejection, as endogenous T cells and NK cells are less likely to recognize the incoming graft as foreign, and are thus less likely to mount an immune response against the transplant.

As used herein, the term "HLA-mismatched" refers to a donor-recipient pair in which at least one HLA antigen, in particular with respect to HLA-A, HLA-B, HLA-C, and HLA-DR, is mismatched between the donor and recipient, such as a donor providing a hematopoietic stem cell graft to a recipient in need of hematopoietic stem cell transplant therapy. In some embodiments, one haplotype is matched and the other is mismatched. HLA-mismatched donor-recipient pairs may have an increased risk of graft rejection relative to HLA-matched donor-recipient pairs, as endogenous T cells and NK cells are more likely to recognize the incoming graft as foreign in the case of an HLA-mismatched donor-recipient pair, and such T cells and NK cells are thus more likely to mount an immune response against the transplant.

As used herein, the terms "induced pluripotent stem cell," "iPS cell," and "iPSC" refer to a pluripotent stem cell that can be derived directly from a differentiated somatic cell. Human iPS cells can be generated by introducing specific sets of reprogramming factors into a non-pluripotent cell that can include, for example, Oct3/4, Sox family transcription factors (e.g., Sox1, Sox2, Sox3, Sox15), Myc family transcription factors (e.g., c-Myc, 1-Myc, n-Myc), Kruppel-like family (KLF) transcription factors (e.g., KLF1, KLF2, KLF4, KLF5), and/or related transcription factors, such as NANOG, LIN28, and/or Glis1. Human iPS cells can also be generated, for example, by the use of miRNAs, small molecules that mimic the actions of transcription factors, or lineage specifiers. Human iPS cells are characterized by their ability to differentiate into any cell of the three vertebrate germ layers, e.g., the endoderm, the ectoderm, or the mesoderm. Human iPS cells are also characterized by their ability to propagate indefinitely under suitable in vitro culture conditions. Human iPS cells are described, for example, in Takahashi and Yamanaka, Cell 126:663 (2006), the disclosure of which is incorporated herein by reference as it pertains to the structure and functionality of iPS cells.

As used herein, the term "inhibitor" refers to an agent (e.g., a small molecule, peptide fragment, protein, antibody, or antigen-binding fragment thereof) that binds to, and/or otherwise suppresses the activity of, a target molecule.

As used herein in the context of hematopoietic stem and/or progenitor cells, the term "mobilization" refers to release of such cells from a stem cell niche where the cells typically reside (e.g., the bone marrow) into peripheral circulation. "Mobilization agents" are agents that are capable of inducing the release of hematopoietic stem and/or progenitor cells from a stem cell niche into peripheral circulation.

As used herein, the term "myeloablative" or "myeloablation" refers to a conditioning regiment that substantially impairs or destroys the hematopoietic system, typically by exposure to a cytotoxic agent or radiation. Myeloablation encompasses complete myeloablation brought on by high doses of cytotoxic agent or total body irradiation that destroys the hematopoietic system.

As used herein, the term "non-myeloablative" or "myelosuppressive" refers to a conditioning regiment that does not eliminate substantially all hematopoietic cells of host origin.

"Percent (%) sequence identity" with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

$$100 \text{ multiplied by } \left(\frac{\text{the fraction } X/Y}{1} \right)$$

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions and/or dosage forms, which are suitable for contact with the tissues of a subject, such as a mammal (e.g., a human) without excessive toxicity, irritation, allergic response and other problem complications commensurate with a reasonable benefit/risk ratio.

As used herein, the term "pharmaceutical composition" refers to a composition containing a therapeutic agent (e.g., an agent that increases C1-INH activity and/or expression to physiologically normal levels) that may be administered to a subject, such as a mammal, e.g., a human, in order to prevent, treat or control a particular disease or condition affecting the mammal, such as HAE as described herein.

As used herein, the term "poloxamer" refers to a non-ionic triblock copolymer composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxyethylene. Poloxamers are also known by the trade name of "Pluronic" or "Synperonics" (BASF). The block copolymer can be represented by the following formula: $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$. The lengths of the polymer blocks can be customized. As a result, many different poloxamers exist. Poloxamers suitable for use in conjunction with the compositions and methods of the present disclosure include those having an average molecular weight of at least about 10,000 g/mol, at least about 11,400 g/mol, at least about 12,600 g/mol, at least about 13,000 g/mol, at least about 14,600 g/mol, or at least about 15,000 g/mol. Since the synthesis of block copolymers is associated with a natural degree of variation from one batch to another, the numerical values recited above (and those used herein to characterize a given poloxamer) may not be precisely achievable upon synthesis, and the average value will differ to a certain extent. Thus, the term "poloxamer" as used herein can be used interchangeably with the term "poloxamers" (representing an entity of several poloxamers, also referred to as mixture of poloxamers) if not explicitly stated otherwise. The term "average" in relation to the number of monomer units or molecular weight of (a) poloxamer(s) as used herein is a consequence of the technical inability to produce poloxamers all having the identical composition and thus the identical molecular weight. Poloxamers produced according to state-of-the-art methods will be present as a mixture of poloxamers each showing a variability as regards their molecular weight, but the mixture as a whole averaging the molecular weight specified herein. BASF and Sigma Aldrich are suitable sources of poloxamers for use in conjunction with the compositions and methods of the disclosure.

As used herein, the term "pluripotent cell" refers to a cell that possesses the ability to develop into more than one differentiated cell type, such as a cell type of the hematopoietic lineage (e.g., granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells). Examples of pluripotent cells are ESCs, iPSCs, and CD34+ cells.

As used herein, the term "promoter" refers to a recognition site on DNA that is bound by an RNA polymerase. The polymerase drives transcription of the transgene. Exemplary promoters suitable for use with the compositions and methods described herein are described, for example, in Sandelin et al., *Nature Reviews Genetics* 8:424 (2007), the disclosure of which is incorporated herein by reference as it pertains to nucleic acid regulatory elements. Additionally, the term "promoter" may refer to a synthetic promoter, which are regulatory DNA sequences that do not occur naturally in biological systems. Synthetic promoters contain parts of naturally occurring promoters combined with

polynucleotide sequences that do not occur in nature and can be optimized to express recombinant DNA using a variety of transgenes, vectors, and target cell types.

As used herein, the term "tissue-specific promoter" refers to a promoter that selectively facilitates the expression of a gene of interest in a particular cell type or tissue type. Examples of tissue-specific promoters that may be used in conjunction with the compositions and methods of the disclosure include a sp146/p47 promoter, CD11b promoter, CD68 promoter, and a sp146/gp9 promoter, among others.

As used herein, the term "ubiquitous promoter" refers to a promoter that facilitates the expression of a gene of interest in a variety of cell types or tissue types, such as a promoter that does not exhibit a preference for facilitating gene expression in one cell type over another or in one tissue type over another. Examples of tissue-specific promoters that may be used in conjunction with the compositions and methods of the disclosure include an elongation factor 1-alpha promoter, among others.

As used herein, the term "plasmid" refers to a to an extrachromosomal circular double stranded DNA molecule into which additional DNA segments may be ligated. A plasmid is a type of vector, a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Certain plasmids are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial plasmids having a bacterial origin of replication and episomal mammalian plasmids). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Certain plasmids are capable of directing the expression of genes to which they are operably linked.

As used herein, a therapeutic agent is considered to be "provided" to a patient if the patient is directly administered the therapeutic agent or if the patient is administered a substance that is processed or metabolized in vivo so as to yield the therapeutic agent endogenously. For example, a patient, such as a patient having HAE as described herein, may be provided a protein of the disclosure (e.g., functional C1-INH) by direct administration of the protein or by administration of a substance (e.g., a C1-INH gene) that is processed or metabolized in vivo so as to yield the desired protein endogenously. Additional examples of "providing" a protein of interest to a patient are instances in which the patient is administered (i) a nucleic acid molecule encoding the protein of interest, (ii) a vector (e.g., a viral vector) containing such a nucleic acid molecule, (iii) a cell or population of cells containing such a vector or nucleic acid molecule, (iv) an interfering RNA molecule, such as a siRNA, shRNA, or miRNA molecule, that stimulates expression of the protein endogenously upon administration to the patient, or (v) a protein precursor that is processed, for example, by way of one or more post-translational modifications, to yield the desired protein endogenously.

As used herein, the term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the gene(s). Such regulatory sequences are described, for example, in Perdew et al., Regulation of Gene Expression (Humana Press, New York, NY, (2014)); incorporated herein by reference.

As used herein, the term "sample" refers to a specimen (e.g., blood, blood component (e.g., serum or plasma), urine, saliva, amniotic fluid, cerebrospinal fluid, tissue (e.g., placental or dermal), pancreatic fluid, chorionic villus sample, and cells) isolated from a subject. The term sample can also relate to a prepared or processed samples, such as a mRNA- or cDNA-containing sample.

5 As used herein, the term "splice variant" refers to a transcribed product (i.e., RNA) of a single gene that can be processed to produce different mRNA molecules as a result of alternative inclusion or exclusion of specific exons (e.g., exon skipping) within the precursor mRNA. Proteins produced from translation of specific splice variants may differ in their structure and biological activity.

 As used herein, the terms "stem cell" and "undifferentiated cell" refer to a cell in an
10 undifferentiated or partially differentiated state that has the developmental potential to differentiate into multiple cell types. A stem cell is capable of proliferation and giving rise to more such stem cells while maintaining its functional potential. Stem cells can divide asymmetrically, which is known as obligatory asymmetrical differentiation, with one daughter cell retaining the functional potential of the parent stem cell and the other daughter cell expressing some distinct other specific function,
15 phenotype and/or developmental potential from the parent cell. The daughter cells themselves can be induced to proliferate and produce progeny that subsequently differentiate into one or more mature cell types, while also retaining one or more cells with parental developmental potential. A differentiated cell may derive from a multipotent cell, which itself is derived from a multipotent cell, and so on. Alternatively, some of the stem cells in a population can divide symmetrically into two stem
20 cells. Accordingly, the term "stem cell" refers to any subset of cells that have the developmental potential, under particular circumstances, to differentiate to a more specialized or differentiated phenotype, and which retain the capacity, under certain circumstances, to proliferate without substantially differentiating. In some embodiments, the term stem cell refers generally to a naturally occurring parent cell whose descendants (progeny cells) specialize, often in different directions, by
25 differentiation, e.g., by acquiring completely individual characters, as occurs in progressive diversification of embryonic cells and tissues. Some differentiated cells also have the capacity to give rise to cells of greater developmental potential. Such capacity may be natural or may be induced artificially upon treatment with various factors. Cells that begin as stem cells might proceed toward a differentiated phenotype, but then can be induced to "reverse" and re-express the stem cell
30 phenotype, a term often referred to as "dedifferentiation" or "reprogramming" or "retrodifferentiation" by persons of ordinary skill in the art.

 As used herein, the term "transgene" refers to a recombinant nucleic acid (e.g., DNA or cDNA) encoding a gene product (e.g., a gene product described herein). The gene product may be an RNA, peptide, or protein. In addition to the coding region for the gene product, the transgene may
35 include or be operably linked to one or more elements to facilitate or enhance expression, such as a promoter, enhancer(s), destabilizing domain(s), response element(s), reporter element(s), insulator element(s), polyadenylation signal(s), and/or other functional elements. Embodiments of the disclosure may utilize any known suitable promoter, enhancer(s), destabilizing domain(s), response element(s), reporter element(s), insulator element(s), polyadenylation signal(s), and/or other
40 functional elements.

As used herein, the term "transfection" refers to any of a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, lipofection, calcium- phosphate precipitation, DEAE- dextran transfection, Nucleofection, squeeze-poration, sonoporation, optical transfection, Magnetofection, impalefection, and the like.

As used herein, the terms "subject" and "patient" are used interchangeably and refer to an organism (e.g., a mammal, such as a human) that is at risk of developing or has been diagnosed as having, and/or is undergoing treatment for, a disease, such as HAE as described herein.

As used herein, the terms "transduction" and "transduce" refer to a method of introducing a viral vector construct or a part thereof into a cell and subsequent expression of a transgene encoded by the vector construct or part thereof in the cell.

As used herein, "treatment" and "treating" refer to an approach for obtaining beneficial or desired results, e.g., clinical results. Beneficial or desired results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions; diminishment of extent of disease or condition; stabilized (i.e., not worsening) state of disease, disorder, or condition; preventing spread of disease or condition; delay or slowing the progress of the disease or condition; amelioration or palliation of the disease or condition; and remission (whether partial or total), whether detectable or undetectable. "Ameliorating" or "palliating" a disease or condition means that the extent and/or undesirable clinical manifestations of the disease, disorder, or condition are lessened and/or time course of the progression is slowed or lengthened, as compared to the extent or time course in the absence of treatment. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder, as well as those prone to or at risk of developing the condition or disorder, as well as those in which the condition or disorder is to be prevented.

As used herein, the term "vector" includes a nucleic acid vector, e.g., a DNA vector, such as a plasmid, an RNA vector, virus, or other suitable replicon (e.g., viral vector). A variety of vectors have been developed for the delivery of polynucleotides encoding exogenous proteins into a prokaryotic or eukaryotic cell. Examples of such expression vectors are disclosed in, e.g., WO 1994/011026; incorporated herein by reference as it pertains to vectors suitable for the expression of a gene of interest. Expression vectors suitable for use with the compositions and methods described herein contain a polynucleotide sequence as well as, e.g., additional sequence elements used for the expression of proteins and/or the integration of these polynucleotide sequences into the genome of a mammalian cell. Vectors that can be used for the expression of a protein or proteins described herein include plasmids that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Additionally, useful vectors for expression of a protein or proteins described herein may contain polynucleotide sequences that enhance the rate of translation of the corresponding gene or genes or improve the stability or nuclear export of the mRNA that results from gene transcription. Examples of such sequence elements are 5' and 3' untranslated regions, an IRES, and a polyadenylation signal site in order to direct efficient transcription of a gene or genes carried on an expression vector. Expression vectors suitable for use with the compositions and

methods described herein may also contain a polynucleotide encoding a marker for selection of cells that contain such a vector. Examples of a suitable marker are genes that encode resistance to antibiotics, such as ampicillin, chloramphenicol, kanamycin, nourseothricin, or zeocin, among others.

As used herein in the context of providing a therapeutic agent to a patient (e.g., a patient having HAE), the terms “C1 esterase inhibitor” its abbreviation, “C1-INH,” “C1 inhibitor,” and “SERPING1” are used interchangeably and refer to the gene encoding C1-INH, or the corresponding protein product, as context will dictate. The terms “C1 esterase inhibitor” its abbreviation, “C1-INH,” “C1 inhibitor,” and “SERPING1” embrace wild-type forms of the C1-INH gene or protein, as well as variants (e.g., splice variants, truncations, concatemers, and fusion constructs, among others) of wild-type C1-INH proteins and nucleic acids encoding the same.

As used herein, the term “functional C1-INH” refers to a wild-type form of the C1-INH gene or protein, as well as variants (e.g., splice variants, truncations, concatemers, and fusion constructs, among others) of wild-type C1-INH proteins and nucleic acids encoding the same, so long as such variants retain normal, physiological abilities of wild-type C1-INH, such as the ability to inhibit C1 esterase. Examples of such variants may include proteins having at least 70% sequence identity (e.g., 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to any of the amino acid sequences of a wild-type C1-INH protein (e.g., SEQ ID NO: 2), such as variants having an amino acid sequence that differs from that of wild-type C1-INH by way of one or more conservative amino acid substitutions, provided that the C1-INH variant retains the therapeutic function of a wild-type C1-INH.

SEQ ID NO: 2 corresponds to UniProt reference sequence P05155, and is shown below:

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MASRLTLLTLLLLLAGDRASSNP NATSSSSQDPESLQDRGEGKVATTVISKMLFVEPILEVSSLPTNSTNSA
25  TKITANTTDEPTTQPTTEPTTQPTIQPTQPTTQLPTDSPTQPTTGSFCPGPVTLCSDLSESHSTEAVLGDALVDFS
    LKLYHAFSAMKKVETNMAFSFPIASLLTQVLLGAGENTKTNLESILSYPKDFTCVHQALKGFTTKGVTSVSQIF
    HSPDLAIRDTFVNASRTLYSSSPRVLSNNSDANLELINTWVAKNTNNKISRLLDSLPSDTRLVLLNAIYLSAKWK
    TTFDPKKT RMEPFHFKNSVIKVPMMNSKKYPVAHFIDQTLKAKVGQLQLSHNLSLVILVLPQNLKRLLEDMEQALS
    PSVFKAIMEKLEMSKFQPTLLTLPRIKVTTSDMLSIMEKLEFFDFS YDLNLCGLTEDPDLQVSAMQHQT VLELT
30  ETGVEAAAASAI SVARTLLVFEVQQPFLEVLWDQ QHKFPVFMGRVYDPRA
    (SEQ ID NO: 2)

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An exemplary C1-INH nucleic acid sequence is GenBank sequence NM_000062.3, which corresponds to SEQ ID NO: 1, shown below:

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35  ATGGCCTCCAGGCTGACCCTGCTGACCCTCCTGCTGCTGCTGCTGGCTGGGGATAGAGCCTCCTCAAATCCAAAT
    GCTACCAGCTCCAGCTCCAGGATCCAGAGAGTTTGCAAGACAGAGGCGAAGGAAGGTCGCAACAACAGTTATC
    TCCAAGATGCTATTCTGTTGAACCCATCCTGGAGGTTTCAGCTTGCCGACAACCAACTCAACAACCAATTAGCC
    ACCAAAATAACAGCTAATACCACTGATGAACCCACCACACAACCCACCACAGAGCCACCACCAACCCACCATC
40  CAACCCACCAACCAACTACCCAGCTCCCAACAGATTCTCCTACCCAGCCCACTACTGGGTCTTCTGCCAGGA
    CCTGTTACTCTCTGCTCTGACTTGGAGAGTCATTCAACAGAGGCCGTGTTGGGGGATGCTTTGGTAGATTCTCC

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CTGAAGCTCTACCACGCCTTCTCAGCAATGAAGAAGGTGGAGACCAACATGGCCTTTTCCCCATTGAGCATCGCC
 AGCCTCCTTACCCAGGTCTGCTCGGGGCTGGGGAGAACACCAAAACAAACCTGGAGAGCATCCTCTCTTACCCC
 AAGGACTTCACCTGTGTCCACCAGGCCCTGAAGGGCTTCACGACCAAAGGTGTACCTCAGTCTCTCAGATCTTC
 CACAGCCCAGACCTGGCCATAAGGGACACCTTTGTGAATGCCTCTCGGACCCGTGTACAGCAGCAGCCCCAGAGTC
 5 CTAAGCAACAACAGTGACGCCAACTTGGAGCTCATCAACACCTGGGTGGCCAAGAACACCAACAACAAGATCAGC
 CGGCTGCTAGACAGTCTGCCCTCCGATACCCGCCTTGTCTCTCAATGCTATCTACCTGAGTGCCAAGTGAAG
 ACAACATTTGATCCCAAGAAAACAGAATGGAACCCCTTCACTTCAAAAACCTCAGTTATAAAAGTGCCCATGATG
 AATAGCAAGAAGTACCTGTGGCCCATTTCAATTGACCAAACCTTTGAAAGCCAAGGTGGGGCAGCTGCAGCTCTCC
 CACAATCTGAGTTTGGTGATCCTGGTACCCAGAACCTGAAACATCGTCTTGAAGACATGGAACAGGCTCTCAGC
 10 CCTTCTGTTTTCAAGGCCATCATGGAGAACTGGAGATGTCCAAGTTCAGCCCACTCTCCTAACACTACCCCGC
 ATCAAAGTGACGACCAGCCAGGATATGCTCTCAATCATGGAGAAATTGGAATCTTCGATTTTTCTTATGACCTT
 AACCTGTGTGGGCTGACAGAGGACCCAGATCTTCAGGTTTCTGCGATGCAGCACCAGACAGTGCTGGAAC TGACA
 GAGACTGGGGTGGAGGCGGCTGCAGCCTCCGCCATCTCTGTGGCCCGCACCTGCTGGTCTTTGAAGTGCAGCAG
 CCCTTCCTCTTCGTGCTCTGGGACCAGCAGCACAAGTTCCTGTCTTCATGGGGCGAGTATATGACCCCAGGGCC
 15 (SEQ ID NO: 1)

As used herein, an agent that "increases expression and/or activity of C1-INH" refers to an agent that, upon administration to a patient (e.g., a human patient having HAE as described herein) facilitates expression of functional C1-INH at physiologically normal levels. Thus, increased

20 expression or activity of C1-INH is relative to the amount present in the patient before treatment with the agent. For example, an agent that "increases expression and/or activity of C1-INH" includes one that, upon administration to a human patient having HAE as described herein, effectuates expression of functional C1-INH at a level of from about 20% to about 200% of functional C1-INH expression observed in a human subject of comparable age and body mass index that does not have HAE. The

25 agent may, for example, effectuate expression of functional C1-INH at a level of about 20% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 30% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 40%

30 of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 50% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 60% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 70% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 80% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional

35 C1-INH at a level of about 90% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of

40 C1-INH at a level of about 90% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of

functional C1-INH at a level of about 100% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 110% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 120% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 130% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 140% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 150% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 160% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 170% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 180% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 190% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of about 200% of that observed in a human subject of comparable age and body mass index that does not have HAE. In some embodiments, the agent effectuates expression of functional C1-INH at a level of more than about 200% (e.g., 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, or more) of that observed in a human subject of comparable age and body mass index that does not have HAE.

As used herein, an agent that "increases expression and/or activity of C1-INH" is preferably not one that will stimulate functional C1-INH expression in a manner sufficiently excessive to induce pathology. For example, an agent that "increases expression and/or activity of C1-INH" is desirably one that recapitulates physiologically normal levels of functional C1-INH expression in a patient (e.g., a human patient having HAE) that has a C1-INH deficiency.

As used herein, the term "alkyl" refers to monovalent, optionally branched alkyl groups, such as those having from 1 to 6 carbon atoms, or more. This term is exemplified by groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-hexyl and the like.

As used herein, the term "lower alkyl" refers to alkyl groups having from 1 to 6 carbon atoms.

As used herein, the term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl). Preferred aryl include phenyl, naphthyl, phenanthrenyl and the like.

As used herein, the terms "alkaryl" and "aryl alkyl" are used interchangeably and refer to an alkyl group containing an aryl moiety. Similarly, the terms "aryl lower alkyl" and the like refer to lower alkyl groups containing an aryl moiety.

As used herein, the term "alkyl aryl" refers to alkyl groups having an aryl substituent, including
5 benzyl, phenethyl and the like.

As used herein, the term "heteroaryl" refers to a monocyclic heteroaromatic, or a bicyclic or a tricyclic fused-ring heteroaromatic group. Particular examples of heteroaromatic groups include optionally substituted pyridyl, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, [2,3-dihydro]benzofuryl, isobenzofuryl, benzothienyl, benzotriazolyl, isobenzothienyl, indolyl, isoindolyl, 3H-indolyl, benzimidazolyl, imidazo[1,2-a]pyridyl, benzothiazolyl, benzoxazolyl, quinoliziny, quinazolinyl, pthalazinyl, quinoxalinyl, cinnolinyl, naphthyridinyl, pyrido[3,4-b]pyridyl, pyrido[3,2-b]pyridyl, pyrido[4,3-b]pyridyl, quinolyl, isoquinolyl, tetrazolyl, 5,6,7,8-tetrahydroquinolyl, 5,6,7,8-tetrahydroisoquinolyl,
10 purinyl, pteridinyl, carbazolyl, xanthenyl, benzoquinolyl, and the like.

As used herein, the term "alkyl heteroaryl" refers to alkyl groups having a heteroaryl substituent, including 2-furylmethyl, 2-thienylmethyl, 2-(1H-indol-3-yl)ethyl and the like.

As used herein, the term "lower alkenyl" refers to alkenyl groups preferably having from 2 to 6 carbon atoms and having at least 1 or 2 sites of alkenyl unsaturation. Exemplary alkenyl groups are
20 ethenyl ($-\text{CH}=\text{CH}_2$), n-2-propenyl (allyl, $-\text{CH}_2\text{CH}=\text{CH}_2$) and the like.

As used herein, the term "alkenyl aryl" refers to alkenyl groups having an aryl substituent, including 2-phenylvinyl and the like.

As used herein, the term "alkenyl heteroaryl" refers to alkenyl groups having a heteroaryl substituent, including 2-(3-pyridinyl)vinyl and the like.

As used herein, the term "lower alkynyl" refers to alkynyl groups preferably having from 2 to 6 carbon atoms and having at least 1-2 sites of alkynyl unsaturation, preferred alkynyl groups include ethynyl ($-\text{C}\equiv\text{CH}$), propargyl ($-\text{CH}_2\text{C}\equiv\text{CH}$), and the like.

As used herein, the term "alkynyl aryl" refers to alkynyl groups having an aryl substituent, including phenylethynyl and the like.

As used herein, the term "alkynyl heteroaryl" refers to alkynyl groups having a heteroaryl substituent, including 2-thienylethynyl and the like.

As used herein, the term "cycloalkyl" refers to a monocyclic cycloalkyl group having from 3 to 8 carbon atoms, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like.

As used herein, the term "lower cycloalkyl" refers to a saturated carbocyclic group of from 3 to 8 carbon atoms having a single ring (e.g., cyclohexyl) or multiple condensed rings (e.g., norbornyl). Preferred cycloalkyl include cyclopentyl, cyclohexyl, norbornyl and the like.

As used herein, the term "heterocycloalkyl" refers to a cycloalkyl group in which one or more ring carbon atoms are replaced with a heteroatom, such as a nitrogen atom, an oxygen atom, a sulfur atom, and the like. Exemplary heterocycloalkyl groups are pyrrolidinyl, piperidinyl, oxopiperidinyl,
40

morpholinyl, piperazinyl, oxopiperazinyl, thiomorpholinyl, azepanyl, diazepanyl, oxazepanyl, thiazepanyl, dioxothiazepanyl, azokanyl, tetrahydrofuranyl, tetrahydropyranyl, and the like.

As used herein, the term "alkyl cycloalkyl" refers to alkyl groups having a cycloalkyl substituent, including cyclohexylmethyl, cyclopentylpropyl, and the like.

5 As used herein, the term "alkyl heterocycloalkyl" refers to C₁-C₆-alkyl groups having a heterocycloalkyl substituent, including 2-(1-pyrrolidinyl)ethyl, 4-morpholinylmethyl, (1-methyl-4-piperidinyl)methyl and the like.

As used herein, the term "carboxy" refers to the group -C(O)OH.

10 As used herein, the term "alkyl carboxy" refers to C₁-C₅-alkyl groups having a carboxy substituent, including 2-carboxyethyl and the like.

As used herein, the term "acyl" refers to the group -C(O)R, wherein R may be, for example, C₁-C₆-alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, or C₁-C₆-alkyl heteroaryl, among other substituents.

15 As used herein, the term "acyloxy" refers to the group -OC(O)R, wherein R may be, for example, C₁-C₆-alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, or C₁-C₆-alkyl heteroaryl, among other substituents.

As used herein, the term "alkoxy" refers to the group -O-R, wherein R is, for example, an optionally substituted alkyl group, such as an optionally substituted C₁-C₆-alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, or C₁-C₆-alkyl heteroaryl, among other substituents. Exemplary alkoxy groups include by way of example, methoxy, ethoxy, phenoxy, and the like.

20 As used herein, the term "alkoxycarbonyl" refers to the group -C(O)OR, wherein R is, for example, hydrogen, C₁-C₆-alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, or C₁-C₆-alkyl heteroaryl, among other possible substituents.

As used herein, the term "alkyl alkoxycarbonyl" refers to alkyl groups having an alkoxycarbonyl substituent, including 2-(benzyloxycarbonyl)ethyl and the like.

25 As used herein, the term "aminocarbonyl" refers to the group -C(O)NRR', wherein each of R and R' may independently be, for example, hydrogen, C₁-C₆-alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, or C₁-C₆-alkyl heteroaryl, among other substituents.

As used herein, the term "alkyl aminocarbonyl" refers to alkyl groups having an aminocarbonyl substituent, including 2-(dimethylaminocarbonyl)ethyl and the like.

30 As used herein, the term "acylamino" refers to the group -NRC(O)R', wherein each of R and R' may independently be, for example, hydrogen, C₁-C₆-alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, or C₁-C₆-alkyl heteroaryl, among other substituents.

As used herein, the term "alkyl acylamino" refers to alkyl groups having an acylamino substituent, including 2-(propionylamino)ethyl and the like.

35 As used herein, the term "ureido" refers to the group -NRC(O)NR'R'', wherein each of R, R', and R'' may independently be, for example, hydrogen, C₁-C₆-alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, C₁-C₆-alkyl heteroaryl, cycloalkyl, or heterocycloalkyl, among other substituents. Exemplary ureido groups further include moieties in which R' and R'', together with the nitrogen atom to which they are attached, form a 3-8-membered heterocycloalkyl ring.

As used herein, the term "alkyl ureido" refers to alkyl groups having an ureido substituent, including 2- (N'-methyleureido)ethyl and the like.

As used herein, the term "amino" refers to the group -NRR', wherein each of R and R' may independently be, for example, hydrogen, C₁-C₆- alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, C₁-C₆-alkyl heteroaryl, cycloalkyl, or heterocycloalkyl, among other substituents. Exemplary amino groups further include moieties in which R and R', together with the nitrogen atom to which they are attached, can form a 3-8-membered heterocycloalkyl ring.

As used herein, the term "alkyl amino" refers to alkyl groups having an amino substituent, including 2- (1 -pyrrolidinyl)ethyl and the like.

As used herein, the term "ammonium" refers to a positively charged group -N⁺RR'R", wherein each of R, R', and R" may independently be, for example, C₁-C₆-alkyl, C₁-C₆-alkyl aryl, C₁-C₆-alkyl heteroaryl, cycloalkyl, or heterocycloalkyl, among other substituents. Exemplary ammonium groups further include moieties in which R and R', together with the nitrogen atom to which they are attached, form a 3-8-membered heterocycloalkyl ring.

As used herein, the term "halogen" refers to fluoro, chloro, bromo and iodo atoms.

As used herein, the term "sulfonyloxy" refers to a group -OSO₂-R wherein R is selected from hydrogen, C₁-C₆-alkyl, C₁-C₆-alkyl substituted with halogens, e.g., an -OSO₂-CF₃ group, aryl, heteroaryl, C₁-C₆-alkyl aryl, and C₁-C₆-alkyl heteroaryl.

As used herein, the term "alkyl sulfonyloxy" refers to alkyl groups having a sulfonyloxy substituent, including 2-(methylsulfonyloxy)ethyl and the like.

As used herein, the term "sulfonyl" refers to group "-SO₂-R" wherein R is selected from hydrogen, aryl, heteroaryl, C₁-C₆-alkyl, C₁-C₆-alkyl substituted with halogens, e.g., an -SO₂-CF₃ group, C₁-C₆- alkyl aryl or C₁-C₆-alkyl heteroaryl.

As used herein, the term "alkyl sulfonyl" refers to alkyl groups having a sulfonyl substituent, including 2-(methylsulfonyl)ethyl and the like.

As used herein, the term "sulfinyl" refers to a group "-S(O)-R" wherein R is selected from hydrogen, C₁-C₆-alkyl, C₁-C₆-alkyl substituted with halogens, e.g., a -SO-CF₃ group, aryl, heteroaryl, C₁-C₆- alkyl aryl or C₁-C₆-alkyl heteroaryl.

As used herein, the term "alkyl sulfinyl" refers to C₁-C₅-alkyl groups having a sulfinyl substituent, including 2-(methylsulfinyl)ethyl and the like.

As used herein, the term "sulfanyl" refers to groups -S-R, wherein R is, for example, alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, or C₁-C₆-alkyl heteroaryl, among other substituents. Exemplary sulfanyl groups are methylsulfanyl, ethylsulfanyl, and the like.

As used herein, the term "alkyl sulfanyl" refers to alkyl groups having a sulfanyl substituent, including 2-(ethylsulfanyl)ethyl and the like.

As used hererin, the term "sulfonylamino" refers to a group -NRSO₂-R', wherein each of R and R' may independently be hydrogen, C₁-C₆-alkyl, aryl, heteroaryl, C₁-C₆-alkyl aryl, or C₁-C₆-alkyl heteroaryl, among other substituents.

As used herein, the term "alkyl sulfonylamino" refers to alkyl groups having a sulfonylamino substituent, including 2-(ethylsulfonylamino)ethyl and the like.

Unless otherwise constrained by the definition of the individual substituent, the above set out groups, like "alkyl", "alkenyl", "alkynyl", "aryl" and "heteroaryl" etc. groups can optionally be substituted, for example, with one or more substituents, as valency permits, such as a substituent selected from alkyl (e.g., C₁-C₆-alkyl), alkenyl (e.g., C₂-C₆-alkenyl), alkynyl (e.g., C₂-C₆-alkynyl),
5 cycloalkyl, heterocycloalkyl, alkyl aryl (e.g., C₁-C₆-alkyl aryl), alkyl heteroaryl (e.g., C₁-C₆-alkyl heteroaryl, alkyl cycloalkyl (e.g., C₁-C₆-alkyl cycloalkyl), alkyl heterocycloalkyl (e.g., C₁-C₆-alkyl heterocycloalkyl), amino, ammonium, acyl, acyloxy, acylamino, aminocarbonyl, alkoxy, carbonyl, ureido, aryl, heteroaryl, sulfinyl, sulfonyl, alkoxy, sulfanyl, halogen, carboxy, trihalomethyl, cyano, hydroxy, mercapto, nitro, and the like. In some embodiments, the substitution is one in which
10 neighboring substituents have undergone ring closure, such as situations in which vicinal functional substituents are involved, thus forming, e.g., lactams, lactones, cyclic anhydrides, acetals, thioacetals, and aminals, among others.

As used herein, the term "optionally fused" refers to a cyclic chemical group that may be fused with a ring system, such as cycloalkyl, heterocycloalkyl, aryl, or heteroaryl. Exemplary ring
15 systems that may be fused to an optionally fused chemical group include, e.g., indolyl, isoindolyl, benzofuranyl, isobenzofuranyl, benzothiophenyl, benzoxazolyl, benzothiazolyl, benzoisoxazolyl, benzoisothiazolyl, indazolyl, benzimidazolyl, quinolyl, isoquinolyl, phthalazyl, quinoxalyl, quinoxalyl, cinnolyl, indolizyl, naphthyridyl, pteridyl, indanyl, naphthyl, 1,2,3,4-tetrahydronaphthyl, indolyl, isoindolyl, 2,3,4,5-tetrahydrobenzo[b]oxepinyl, 6,7,8,9-tetrahydro-5H-benzocycloheptenyl, chromanyl, and the like.
20

As used herein, the term "pharmaceutically acceptable salt" refers to a salt, such as a salt of a compound described herein, that retains the desired biological activity of the non-ionized parent compound from which the salt is formed. Examples of such salts include, but are not restricted to acid addition salts formed with inorganic acids (e.g., hydrochloric acid, hydrobromic acid, sulfuric acid,
25 phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, fumaric acid, maleic acid, ascorbic acid, benzoic acid, tannic acid, pantoic acid, alginic acid, polyglutamic acid, naphthalene sulfonic acid, naphthalene disulfonic acid, and poly-galacturonic acid. The compounds can also be administered as pharmaceutically acceptable quaternary salts, such as quaternary ammonium salts of the formula -
30 NR₃⁺Z⁻, wherein each of R, R', and R" may independently be, for example, hydrogen, alkyl, benzyl, C₁-C₆-alkyl, C₂-C₆-alkenyl, C₂-C₆-alkynyl, C₁-C₆-alkyl aryl, C₁-C₆-alkyl heteroaryl, cycloalkyl, heterocycloalkyl, or the like, and Z is a counterion, such as chloride, bromide, iodide, -O-alkyl, toluenesulfonate, methyl sulfonate, sulfonate, phosphate, carboxylate (such as benzoate, succinate, acetate, glycolate, maleate, malate, fumarate, citrate, tartrate, ascorbate, cinnamate, mandelate,
35 and diphenylacetate), or the like.

As used herein, for example, in the context of a protein kinase C (PKC) inhibitor, such as staurosporine, the term "variant" refers to an agent containing one or more modifications relative to a reference agent and that (i) retains a functional property of the reference agent (e.g., the ability to inhibit PKC activity) and/or (ii) is converted within a cell (e.g., a cell of a type described herein, such
40 as a CD34⁺ cell) into the reference agent. In the context of small molecule PKC inhibitors, such as

staurosporine, structural variants of a reference compound include those that differ from the reference compound by the inclusion and/or location of one or more substituents, as well as variants that are isomers of a reference compound, such as structural isomers (e.g., regioisomers) or stereoisomers (e.g., enantiomers or diastereomers), as well as prodrugs of a reference compound. In the context of an interfering RNA molecule, a variant may contain one or more nucleic acid substitutions relative to a parent interfering RNA molecule.

The structural compositions described herein also include the tautomers, geometrical isomers (e.g., E/Z isomers and cis/trans isomers), enantiomers, diastereomers, and racemic forms, as well as pharmaceutically acceptable salts thereof. Such salts include, e.g., acid addition salts formed with pharmaceutically acceptable acids like hydrochloride, hydrobromide, sulfate or bisulfate, phosphate or hydrogen phosphate, acetate, benzoate, succinate, fumarate, maleate, lactate, citrate, tartrate, gluconate, methanesulfonate, benzenesulfonate, and para-toluenesulfonate salts.

As used herein, chemical structural formulas that do not depict the stereochemical configuration of a compound having one or more stereocenters will be interpreted as encompassing any one of the stereoisomers of the indicated compound, or a mixture of one or more such stereoisomers (e.g., any one of the enantiomers or diastereomers of the indicated compound, or a mixture of the enantiomers (e.g., a racemic mixture) or a mixture of the diastereomers). As used herein, chemical structural formulas that do specifically depict the stereochemical configuration of a compound having one or more stereocenters will be interpreted as referring to the substantially pure form of the particular stereoisomer shown. "Substantially pure" forms refer to compounds having a purity of greater than 85%, such as a purity of from 85% to 99%, 85% to 99.9%, 85% to 99.99%, or 85% to 100%, such as a purity of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, or 100%, as assessed, for example, using chromatography and nuclear magnetic resonance techniques known in the art.

Brief Description of the Drawings

FIG. 1 is a schematic drawing showing examples of therapeutic lentiviral vector constructs generated for *ex vivo* HSC transduction. Wild type or codon optimized version of human SERPING1 gene expression is under constitutive Elongation Factor 1 alpha core promoter. SERPING1 coding sequences are combined with a WPRE posttranscriptional regulatory element to enhance expression.

FIGS. 2A and 2B are schematic drawings showing examples of therapeutic lentiviral vectors generated for *in vivo* transduction construct features. Shown are constructs with wild type (FIG. 2A) or codon optimized versions (FIG. 2B) of human SERPING1 under enhanced transthyretin promoter control.

FIGS. 3A and 3B are graphs showing real time RT-PCR evaluating WT SERPING1 gene expression in HT29 cells (FIG. 3A) and K562 cells (FIG. 3B).

FIG. 4 is a gel picture showing western blot analysis of SERPING1 protein levels in whole cell lysates from HT29 cells transduced with LV vectors.

FIG. 5 is a graph showing the results from an ELISA assay used to evaluate functional C1 inhibitor levels in serum-free supernatant from a K562 cell line transduced with LV-SERPING1. HS = human serum.

FIG. 6 is a graph showing the evaluation of endogenous levels of SERPING1 in primary cells and cell lines. Ct = cycle threshold.

FIG. 7A is a graph showing high-efficiency LV transduction of CD34+ cells with codon optimized (CO) and WT SERPING1 LV vectors.

FIG. 7B is a graph showing that LV transduction of CD34+ cells with codon optimized (CO) and WT SERPING1 LV vectors increased expression by ~45fold and ~13fold relative to endogenous SERPING1 gene expression levels.

FIG. 8 is a picture of a gel showing results from a western blot analysis of SERPING1 protein levels in whole cell lysates from CD34+ cells transduced with a LV vector containing a codon optimized SERPING1 transgene. UnTd = untransduced CD34+ cells.

FIGS. 9A-9C are a set of graphs showing that SERPING-1 HSC gene therapy results in significant increase in levels of functional serum C1-inhibitor production in a xenotransplant model. FIG. 9A shows *in vitro* characterization of vector copy number (VCN) and transduction efficiency with LV-SERPING1. FIG. 9B shows analysis of human chimerism in bone marrow of xenografted NSG-SGM3 mice and differentiation of CD11b+CD14+ myeloid subsets *in vitro*. FIG. 9C shows serum levels of human functional C1-inhibitor production. UNTRD = untransduced.

Detailed Description

The present disclosure provides compositions and methods for treating or preventing HAE. The compositions and methods described herein may be used, for example, to treat a patient, such as a child, adolescent, or adult human patient, that is suffering from HAE, as well as to prophylactically treat a patient at risk of developing HAE. Patients may be treated, for example, by providing to the patients one or more agents that elevate the expression and/or activity of functional C1-esterase inhibitor (C1-INH), such as a population of cells (e.g., a population of pluripotent cells, such as hematopoietic stem cells) that express functional C1-INH. Without being limited by mechanism, the provision of such agents may treat an underlying cause of the disease and reverse its pathophysiology. Thus, using the compositions and methods described herein, a patient may not only be treated in a manner that alleviates one or more symptoms associated with HAE, but also in a curative fashion or preventative fashion.

C1-INH Activity

C1-INH is a highly glycosylated protease inhibitor belonging to the serpin superfamily of proteins (Serpin family G member 1). Its main function is the inhibition of the complement system to prevent spontaneous activation and also plays a role as a major regulator of the contact system. C1-INH is an acute-phase protein that circulates in blood and exhibits approximately a two-fold rise during inflammation. C1-INH irreversibly binds to and inactivates C1r and C1s proteases in the C1 complex of classical pathway of complement. MASP-1 and MASP-2 proteases in MBL complexes of

the lectin pathway are also inactivated. C1-inhibitor prevents the proteolytic cleavage of later complement components C4 and C2 by C1 and MBL. C1-inhibitor also inhibits proteases of other pathways, such as the fibrinolytic, clotting, and kinin pathways.

Deficiency of C1-INH (e.g., due to decreased levels of functional protein in the serum or blood plasma) may cause hereditary angioedema, which includes swelling due to leakage of fluid from blood vessels into connection tissue. Deficiency of C1-INH leads to activation of kallikrein within plasma, thereby leading to the production of the vasoactive peptide bradykinin. Moreover, deficiency of C1-INH leads to a lack of inhibition of C4 and C2 cleavage, thereby leading to activation of the complement system, ultimately resulting in the pathogenesis of HAE.

Using the compositions and methods of the disclosure, an agent that increases C1-INH activity and/or expression, such as a viral vector encoding, or a cell (e.g., a CD34+ cell or other pluripotent cell described herein) that expresses, C1-INH can be administered to a patient suffering from HAE (e.g., a patient having a defect in C1-INH expression) so as to promote restoration of physiologically normal levels of C1-INH (e.g., from 15 mg/dl to about 35 mg/dl), normal complement system activity, and reduction in the severity and number of HAE related attacks.

Without being limited by mechanism, the section that follows describes how agents that increase the C1-INH activity and/or expression and effectuate one or more, or all, of the beneficial phenotypes described above can be used to treat HAE.

HAE

Etiology and C1-INH restoration therapy

HAE is a disease that can be caused by defective C1-INH activity. This aberration in C1-INH activity can be triggered by mutations clustered in the reactive center loop (RCL) of C1-INH. Such a mutation includes, e.g., K251. Other deleterious mutations include, e.g., A436T, R444H, R444C, R444S, V432E, A443V, Y199TER, I462S, and R378C. The lack of normal levels functional C1-INH lead to a disruption of the complement pathway. Symptoms typically begin in childhood and worsen through puberty. Untreated subjects typically have an attack every 1 to 2 weeks, and episodes may last for 3 to 4 days.

C1-INH contains a C-terminal inhibitor domain that irreversibly binds to and inactivates the C1r and C1s proteases in the C1 complex. However, mutations in C1-INH, such as those described above, prevent correct protein-protein interactions and inhibition of its downstream targets.

Using the compositions and methods of the disclosure, a patient, such as a human patient suffering from HAE, may be administered an agent that expresses a functional C1-INH protein that does not contain an activity-disrupting mutation. Exemplary agents that achieve this effect are pluripotent cells, such as hematopoietic stem cells and hematopoietic progenitor cells, that express functional C1-INH and/or viral vectors encoding the same. The functional C1-INH may be encoded by the WT sequence or a codon-optimized variant thereof. The sections that follow describe exemplary procedures for producing such agents, as well as how such agents may be used to treat a patient suffering from HAE.

Diagnosis

A patient (e.g., a human patient) can be diagnosed as having HAE in a variety of ways. Genetic testing offers one avenue by which a patient may be diagnosed as having (or at risk of developing) HAE. For example, a genetic analysis can be used to determine whether a patient has a

5 loss-of-function mutation in an endogenous gene encoding C1-INH, such as a mutation in a C1-INH gene selected from the group consisting of: K251, A436T, R444H, R444C, R444S, V432E, A443V, Y199TER, I462S, and R378C. Exemplary genetic tests that can be used to determine whether a patient has such a mutation include polymerase chain reaction (PCR) methods known in the art and described herein, among others.

10 Clinically, HAE may be detected, for example, by way of a blood test. In this setting, HAE may be characterized by an insufficiency or low level of C1-INH (e.g., less than about 7 mg/dl) in blood cells, which can be detected in blood by using routine molecular biology techniques known in the art, such as PCR-based methods, among others. Other proteins, such as C4 or C1q, may be used to diagnose HAE.

15 The patient may be diagnosed by persistence of swelling episodes, such as in the hands, feet, face, or throat (e.g., larynx).

In some embodiments, the patient has previously been treated with one or more therapeutic agents selected from the group consisting of C1-esterase inhibitor (e.g., BERINERT® or RUCONEST®), icatibant (e.g., icatibant injectin, e.g., FIRAZYR®), and ecallantide (e.g.,

20 KALBITOR®). The patient may not have responded to treatment with the one or more therapeutic agents. In some embodiments, the patient has previously been treated with one or more prophylactic agents selected from the group consisting of Cinryze, Haegarda, Takhzyro, and an androgen. The patient may not have responded to treatment with the one or more prophylactic agents.

In some embodiments, the patient is less than 12 years old (e.g., less than 6 years old). In

25 some embodiments, the patient is more than 6 years old (e.g., more than 12 years old). In some embodiments, the patient exhibits angioedema attacks with a frequency of from one to ten times per month (e.g., one or two times per week).

Prevention

30 Using the compositions and methods described herein, a subject (e.g., a human subject) may be administered one or more agents that increase activity and/or expression of functional C1-INH (e.g., to within physiologically normal levels or above physiological levels) so as to prevent the onset of, or frequency of attacks related to, HAE. The subject may be one that is at risk of developing HAE but has not yet presented with an observable symptom of the disease. For example, the subject may

35 be one that has a loss-of-function mutation in an endogenous gene encoding C1-INH, such as a substitution or deletion mutation in a C1-INH gene (e.g., within the reactive center loop of C1-INH). The mutation may be selected from the group consisting of K251, A436T, R444H, R444C, R444S, V432E, A443V, Y199TER, I462S, and R378C. In some embodiments, the patient has a mutation in an endogenous gene encoding C1-INH that causes deletion or expression of a truncated transcript.

40 The patient may have a mutation in a gene encoding coagulation factor XII. The patient may have a

heterozygous or homozygous mutation. As described above, a subject can be identified as having such a mutation using standard molecular biology techniques known in the art and described herein, including PCR-based methodologies, among others.

5 Methods of Producing Functional C1-INH-Expressing Cells by Viral Transduction

Transduction using a poloxamer

Poloxamers may be used in conjunction with the compositions and methods of the disclosure to enhance transduction efficiency. Poloxamers that may be used include those having an average molar mass of polyoxypropylene subunits of greater than 2,050 g/mol (e.g., an average molar mass of polyoxypropylene subunits of about 2,055 g/mol, 2,060 g/mol, 2,075 g/mol, 2,080 g/mol, 2,085 g/mol, 2,090 g/mol, 2,095 g/mol, 2,100 g/mol, 2,200 g/mol, 2,300 g/mol, 2,400 g/mol, 2,500 g/mol, 2,600 g/mol, 2,700 g/mol, 2,800 g/mol, 2,900 g/mol, 3,000 g/mol, 3,100 g/mol, 3,200 g/mol, 3,300 g/mol, 3,400 g/mol, 3,500 g/mol, 3,600 g/mol, 3,700 g/mol, 3,800 g/mol, 3,900 g/mol, 4,000 g/mol, 4,100 g/mol, 4,200 g/mol, 4,300 g/mol, 4,400 g/mol, 4,500 g/mol, 4,600 g/mol, 4,700 g/mol, 4,800 g/mol, 4,900 g/mol, or 5,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of polyoxypropylene subunits of greater than 2,250 g/mol (e.g., an average molar mass of polyoxypropylene subunits of about 2,300 g/mol, 2,400 g/mol, 2,500 g/mol, 2,600 g/mol, 2,700 g/mol, 2,800 g/mol, 2,900 g/mol, 3,000 g/mol, 3,100 g/mol, 3,200 g/mol, 3,300 g/mol, 3,400 g/mol, 3,500 g/mol, 3,600 g/mol, 3,700 g/mol, 3,800 g/mol, 3,900 g/mol, 4,000 g/mol, 4,100 g/mol, 4,200 g/mol, 4,300 g/mol, 4,400 g/mol, 4,500 g/mol, 4,600 g/mol, 4,700 g/mol, 4,800 g/mol, 4,900 g/mol, or 5,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of polyoxypropylene subunits of greater than 2,750 g/mol (e.g., an average molar mass of polyoxypropylene subunits of about 2,800 g/mol, 2,900 g/mol, 3,000 g/mol, 3,100 g/mol, 3,200 g/mol, 3,300 g/mol, 3,400 g/mol, 3,500 g/mol, 3,600 g/mol, 3,700 g/mol, 3,800 g/mol, 3,900 g/mol, 4,000 g/mol, 4,100 g/mol, 4,200 g/mol, 4,300 g/mol, 4,400 g/mol, 4,500 g/mol, 4,600 g/mol, 4,700 g/mol, 4,800 g/mol, 4,900 g/mol, or 5,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of polyoxypropylene subunits of greater than 3,250 g/mol (e.g., an average molar mass of polyoxypropylene subunits of about 3,300 g/mol, 3,400 g/mol, 3,500 g/mol, 3,600 g/mol, 3,700 g/mol, 3,800 g/mol, 3,900 g/mol, 4,000 g/mol, 4,100 g/mol, 4,200 g/mol, 4,300 g/mol, 4,400 g/mol, 4,500 g/mol, 4,600 g/mol, 4,700 g/mol, 4,800 g/mol, 4,900 g/mol, or 5,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of polyoxypropylene subunits of greater than 3,625 g/mol (e.g., an average molar mass of polyoxypropylene subunits of about 3,700 g/mol, 3,800 g/mol, 3,900 g/mol, 4,000 g/mol, 4,100 g/mol, 4,200 g/mol, 4,300 g/mol, 4,400 g/mol, 4,500 g/mol, 4,600 g/mol, 4,700 g/mol, 4,800 g/mol, 4,900 g/mol, or 5,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of polyoxypropylene subunits of from about 2,050 g/mol to about 4,000 g/mol (e.g., about 2,050 g/mol, 2,055 g/mol, 2,060 g/mol, 2,065 g/mol, 2,070 g/mol, 2,075 g/mol, 2,080 g/mol, 2,085 g/mol, 2,090 g/mol, 2,095 g/mol, 2,100 g/mol, 2,105 g/mol, 2,110 g/mol, 2,115 g/mol, 2,120 g/mol, 2,125 g/mol, 2,130 g/mol, 2,135

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40 3,600 g/mol, 3,605 g/mol, 3,610 g/mol, 3,615 g/mol, 3,620 g/mol, 3,625 g/mol, 3,630 g/mol, 3,635

g/mol, 3,640 g/mol, 3,645 g/mol, 3,650 g/mol, 3,655 g/mol, 3,660 g/mol, 3,665 g/mol, 3,670 g/mol, 3,675 g/mol, 3,680 g/mol, 3,685 g/mol, 3,690 g/mol, 3,695 g/mol, 3,700 g/mol, 3,705 g/mol, 3,710 g/mol, 3,715 g/mol, 3,720 g/mol, 3,725 g/mol, 3,730 g/mol, 3,735 g/mol, 3,740 g/mol, 3,745 g/mol, 3,750 g/mol, 3,755 g/mol, 3,760 g/mol, 3,765 g/mol, 3,770 g/mol, 3,775 g/mol, 3,780 g/mol, 3,785 g/mol, 3,790 g/mol, 3,795 g/mol, 3,800 g/mol, 3,805 g/mol, 3,810 g/mol, 3,815 g/mol, 3,820 g/mol, 3,825 g/mol, 3,830 g/mol, 3,835 g/mol, 3,840 g/mol, 3,845 g/mol, 3,850 g/mol, 3,855 g/mol, 3,860 g/mol, 3,865 g/mol, 3,870 g/mol, 3,875 g/mol, 3,880 g/mol, 3,885 g/mol, 3,890 g/mol, 3,895 g/mol, 3,900 g/mol, 3,905 g/mol, 3,910 g/mol, 3,915 g/mol, 3,920 g/mol, 3,925 g/mol, 3,930 g/mol, 3,935 g/mol, 3,940 g/mol, 3,945 g/mol, 3,950 g/mol, 3,955 g/mol, 3,960 g/mol, 3,965 g/mol, 3,970 g/mol, 3,975 g/mol, 3,980 g/mol, 3,985 g/mol, 3,990 g/mol, 3,995 g/mol, or 4,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of polyoxypropylene subunits of from about 2,750 g/mol to about 4,000 g/mol (e.g., about 2,750 g/mol, 2,755 g/mol, 2,760 g/mol, 2,765 g/mol, 2,770 g/mol, 2,775 g/mol, 2,780 g/mol, 2,785 g/mol, 2,790 g/mol, 2,795 g/mol, 2,800 g/mol, 2,805 g/mol, 2,810 g/mol, 2,815 g/mol, 2,820 g/mol, 2,825 g/mol, 2,830 g/mol, 2,835 g/mol, 2,840 g/mol, 2,845 g/mol, 2,850 g/mol, 2,855 g/mol, 2,860 g/mol, 2,865 g/mol, 2,870 g/mol, 2,875 g/mol, 2,880 g/mol, 2,885 g/mol, 2,890 g/mol, 2,895 g/mol, 2,900 g/mol, 2,905 g/mol, 2,910 g/mol, 2,915 g/mol, 2,920 g/mol, 2,925 g/mol, 2,930 g/mol, 2,935 g/mol, 2,940 g/mol, 2,945 g/mol, 2,950 g/mol, 2,955 g/mol, 2,960 g/mol, 2,965 g/mol, 2,970 g/mol, 2,975 g/mol, 2,980 g/mol, 2,985 g/mol, 2,990 g/mol, 2,995 g/mol, 3,000 g/mol, 3,005 g/mol, 3,010 g/mol, 3,015 g/mol, 3,020 g/mol, 3,025 g/mol, 3,030 g/mol, 3,035 g/mol, 3,040 g/mol, 3,045 g/mol, 3,050 g/mol, 3,055 g/mol, 3,060 g/mol, 3,065 g/mol, 3,070 g/mol, 3,075 g/mol, 3,080 g/mol, 3,085 g/mol, 3,090 g/mol, 3,095 g/mol, 3,100 g/mol, 3,105 g/mol, 3,110 g/mol, 3,115 g/mol, 3,120 g/mol, 3,125 g/mol, 3,130 g/mol, 3,135 g/mol, 3,140 g/mol, 3,145 g/mol, 3,150 g/mol, 3,155 g/mol, 3,160 g/mol, 3,165 g/mol, 3,170 g/mol, 3,175 g/mol, 3,180 g/mol, 3,185 g/mol, 3,190 g/mol, 3,195 g/mol, 3,200 g/mol, 3,205 g/mol, 3,210 g/mol, 3,215 g/mol, 3,220 g/mol, 3,225 g/mol, 3,230 g/mol, 3,235 g/mol, 3,240 g/mol, 3,245 g/mol, 3,250 g/mol, 3,255 g/mol, 3,260 g/mol, 3,265 g/mol, 3,270 g/mol, 3,275 g/mol, 3,280 g/mol, 3,285 g/mol, 3,290 g/mol, 3,295 g/mol, 3,300 g/mol, 3,305 g/mol, 3,310 g/mol, 3,315 g/mol, 3,320 g/mol, 3,325 g/mol, 3,330 g/mol, 3,335 g/mol, 3,340 g/mol, 3,345 g/mol, 3,350 g/mol, 3,355 g/mol, 3,360 g/mol, 3,365 g/mol, 3,370 g/mol, 3,375 g/mol, 3,380 g/mol, 3,385 g/mol, 3,390 g/mol, 3,395 g/mol, 3,400 g/mol, 3,405 g/mol, 3,410 g/mol, 3,415 g/mol, 3,420 g/mol, 3,425 g/mol, 3,430 g/mol, 3,435 g/mol, 3,440 g/mol, 3,445 g/mol, 3,450 g/mol, 3,455 g/mol, 3,460 g/mol, 3,465 g/mol, 3,470 g/mol, 3,475 g/mol, 3,480 g/mol, 3,485 g/mol, 3,490 g/mol, 3,495 g/mol, 3,500 g/mol, 3,505 g/mol, 3,510 g/mol, 3,515 g/mol, 3,520 g/mol, 3,525 g/mol, 3,530 g/mol, 3,535 g/mol, 3,540 g/mol, 3,545 g/mol, 3,550 g/mol, 3,555 g/mol, 3,560 g/mol, 3,565 g/mol, 3,570 g/mol, 3,575 g/mol, 3,580 g/mol, 3,585 g/mol, 3,590 g/mol, 3,595 g/mol, 3,600 g/mol, 3,605 g/mol, 3,610 g/mol, 3,615 g/mol, 3,620 g/mol, 3,625 g/mol, 3,630 g/mol, 3,635 g/mol, 3,640 g/mol, 3,645 g/mol, 3,650 g/mol, 3,655 g/mol, 3,660 g/mol, 3,665 g/mol, 3,670 g/mol, 3,675 g/mol, 3,680 g/mol, 3,685 g/mol, 3,690 g/mol, 3,695 g/mol, 3,700 g/mol, 3,705 g/mol, 3,710 g/mol, 3,715 g/mol, 3,720 g/mol, 3,725 g/mol, 3,730 g/mol, 3,735 g/mol, 3,740 g/mol, 3,745 g/mol, 3,750 g/mol, 3,755 g/mol, 3,760 g/mol, 3,765 g/mol, 3,770 g/mol, 3,775 g/mol, 3,780 g/mol, 3,785 g/mol, 3,790 g/mol, 3,795 g/mol, 3,800 g/mol, 3,805 g/mol, 3,810

g/mol, 3,815 g/mol, 3,820 g/mol, 3,825 g/mol, 3,830 g/mol, 3,835 g/mol, 3,840 g/mol, 3,845 g/mol, 3,850 g/mol, 3,855 g/mol, 3,860 g/mol, 3,865 g/mol, 3,870 g/mol, 3,875 g/mol, 3,880 g/mol, 3,885 g/mol, 3,890 g/mol, 3,895 g/mol, 3,900 g/mol, 3,905 g/mol, 3,910 g/mol, 3,915 g/mol, 3,920 g/mol, 3,925 g/mol, 3,930 g/mol, 3,935 g/mol, 3,940 g/mol, 3,945 g/mol, 3,950 g/mol, 3,955 g/mol, 3,960 g/mol, 3,965 g/mol, 3,970 g/mol, 3,975 g/mol, 3,980 g/mol, 3,985 g/mol, 3,990 g/mol, 3,995 g/mol, or 4,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of polyoxypropylene subunits of from about 3,250 g/mol to about 4,000 g/mol (e.g., about 3,250 g/mol, 3,255 g/mol, 3,260 g/mol, 3,265 g/mol, 3,270 g/mol, 3,275 g/mol, 3,280 g/mol, 3,285 g/mol, 3,290 g/mol, 3,295 g/mol, 3,300 g/mol, 3,305 g/mol, 3,310 g/mol, 3,315 g/mol, 3,320 g/mol, 3,325 g/mol, 3,330 g/mol, 3,335 g/mol, 3,340 g/mol, 3,345 g/mol, 3,350 g/mol, 3,355 g/mol, 3,360 g/mol, 3,365 g/mol, 3,370 g/mol, 3,375 g/mol, 3,380 g/mol, 3,385 g/mol, 3,390 g/mol, 3,395 g/mol, 3,400 g/mol, 3,405 g/mol, 3,410 g/mol, 3,415 g/mol, 3,420 g/mol, 3,425 g/mol, 3,430 g/mol, 3,435 g/mol, 3,440 g/mol, 3,445 g/mol, 3,450 g/mol, 3,455 g/mol, 3,460 g/mol, 3,465 g/mol, 3,470 g/mol, 3,475 g/mol, 3,480 g/mol, 3,485 g/mol, 3,490 g/mol, 3,495 g/mol, 3,500 g/mol, 3,505 g/mol, 3,510 g/mol, 3,515 g/mol, 3,520 g/mol, 3,525 g/mol, 3,530 g/mol, 3,535 g/mol, 3,540 g/mol, 3,545 g/mol, 3,550 g/mol, 3,555 g/mol, 3,560 g/mol, 3,565 g/mol, 3,570 g/mol, 3,575 g/mol, 3,580 g/mol, 3,585 g/mol, 3,590 g/mol, 3,595 g/mol, 3,600 g/mol, 3,605 g/mol, 3,610 g/mol, 3,615 g/mol, 3,620 g/mol, 3,625 g/mol, 3,630 g/mol, 3,635 g/mol, 3,640 g/mol, 3,645 g/mol, 3,650 g/mol, 3,655 g/mol, 3,660 g/mol, 3,665 g/mol, 3,670 g/mol, 3,675 g/mol, 3,680 g/mol, 3,685 g/mol, 3,690 g/mol, 3,695 g/mol, 3,700 g/mol, 3,705 g/mol, 3,710 g/mol, 3,715 g/mol, 3,720 g/mol, 3,725 g/mol, 3,730 g/mol, 3,735 g/mol, 3,740 g/mol, 3,745 g/mol, 3,750 g/mol, 3,755 g/mol, 3,760 g/mol, 3,765 g/mol, 3,770 g/mol, 3,775 g/mol, 3,780 g/mol, 3,785 g/mol, 3,790 g/mol, 3,795 g/mol, 3,800 g/mol, 3,805 g/mol, 3,810 g/mol, 3,815 g/mol, 3,820 g/mol, 3,825 g/mol, 3,830 g/mol, 3,835 g/mol, 3,840 g/mol, 3,845 g/mol, 3,850 g/mol, 3,855 g/mol, 3,860 g/mol, 3,865 g/mol, 3,870 g/mol, 3,875 g/mol, 3,880 g/mol, 3,885 g/mol, 3,890 g/mol, 3,895 g/mol, 3,900 g/mol, 3,905 g/mol, 3,910 g/mol, 3,915 g/mol, 3,920 g/mol, 3,925 g/mol, 3,930 g/mol, 3,935 g/mol, 3,940 g/mol, 3,945 g/mol, 3,950 g/mol, 3,955 g/mol, 3,960 g/mol, 3,965 g/mol, 3,970 g/mol, 3,975 g/mol, 3,980 g/mol, 3,985 g/mol, 3,990 g/mol, 3,995 g/mol, or 4,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of polyoxypropylene subunits of from about 3,625 g/mol to about 4,000 g/mol (e.g., about 3,625 g/mol, 3,630 g/mol, 3,635 g/mol, 3,640 g/mol, 3,645 g/mol, 3,650 g/mol, 3,655 g/mol, 3,660 g/mol, 3,665 g/mol, 3,670 g/mol, 3,675 g/mol, 3,680 g/mol, 3,685 g/mol, 3,690 g/mol, 3,695 g/mol, 3,700 g/mol, 3,705 g/mol, 3,710 g/mol, 3,715 g/mol, 3,720 g/mol, 3,725 g/mol, 3,730 g/mol, 3,735 g/mol, 3,740 g/mol, 3,745 g/mol, 3,750 g/mol, 3,755 g/mol, 3,760 g/mol, 3,765 g/mol, 3,770 g/mol, 3,775 g/mol, 3,780 g/mol, 3,785 g/mol, 3,790 g/mol, 3,795 g/mol, 3,800 g/mol, 3,805 g/mol, 3,810 g/mol, 3,815 g/mol, 3,820 g/mol, 3,825 g/mol, 3,830 g/mol, 3,835 g/mol, 3,840 g/mol, 3,845 g/mol, 3,850 g/mol, 3,855 g/mol, 3,860 g/mol, 3,865 g/mol, 3,870 g/mol, 3,875 g/mol, 3,880 g/mol, 3,885 g/mol, 3,890 g/mol, 3,895 g/mol, 3,900 g/mol, 3,905 g/mol, 3,910 g/mol, 3,915 g/mol, 3,920 g/mol, 3,925 g/mol, 3,930 g/mol, 3,935 g/mol, 3,940 g/mol, 3,945 g/mol, 3,950 g/mol, 3,955 g/mol, 3,960 g/mol, 3,965 g/mol, 3,970 g/mol, 3,975 g/mol, 3,980 g/mol, 3,985 g/mol, 3,990 g/mol, 3,995 g/mol, or 4,000 g/mol).

In some embodiments, the poloxamer has an average ethylene oxide content of greater than 40% by mass (e.g., about 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, or more).

In some embodiments, the poloxamer has an average ethylene oxide content of greater than 50% by mass (e.g., about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, or more).

In some embodiments, the poloxamer has an average ethylene oxide content of greater than 60% by mass (e.g., about 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, or more).

In some embodiments, the poloxamer has an average ethylene oxide content of greater than 70% by mass (e.g., about 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, or more).

In some embodiments, the poloxamer has an average ethylene oxide content of from about 40% to about 90% (e.g., about 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%).

In some embodiments, the poloxamer has an average ethylene oxide content of from about 50% to about 85% (e.g., about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, or 85%).

In some embodiments, the poloxamer has an average ethylene oxide content of from about 60% to about 80% (e.g., about 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%).

In some embodiments, the poloxamer has an average molar mass of greater than 10,000 g/mol (e.g., about 10,100 g/mol, 10,200 g/mol, 10,300 g/mol, 10,400 g/mol, 10,500 g/mol, 10,600 g/mol, 10,700 g/mol, 10,800 g/mol, 10,900 g/mol, 11,000 g/mol, 11,100 g/mol, 11,200 g/mol, 11,300 g/mol, 11,400 g/mol, 11,500 g/mol, 11,600 g/mol, 11,700 g/mol, 11,800 g/mol, 11,900 g/mol, 12,000 g/mol, 12,100 g/mol, 12,200 g/mol, 12,300 g/mol, 12,400 g/mol, 12,500 g/mol, 12,600 g/mol, 12,700 g/mol, 12,800 g/mol, 12,900 g/mol, 13,000 g/mol, 13,100 g/mol, 13,200 g/mol, 13,300 g/mol, 13,400 g/mol, 13,500 g/mol, 13,600 g/mol, 13,700 g/mol, 13,800 g/mol, 13,900 g/mol, 14,000 g/mol, 14,100 g/mol, 14,200 g/mol, 14,300 g/mol, 14,400 g/mol, 14,500 g/mol, 14,600 g/mol, 14,700 g/mol, 14,800 g/mol, 14,900 g/mol, or 15,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of greater than 11,000 g/mol (e.g., about 11,100 g/mol, 11,200 g/mol, 11,300 g/mol, 11,400 g/mol, 11,500 g/mol, 11,600

In some embodiments, the poloxamer has an average molar mass of greater than 12,000 g/mol (e.g., about 12,100 g/mol, 12,200 g/mol, 12,300 g/mol, 12,400 g/mol, 12,500 g/mol, 12,600 g/mol, 12,700 g/mol, 12,800 g/mol, 12,900 g/mol, 13,000 g/mol, 13,100 g/mol, 13,200 g/mol, 13,300 g/mol, 13,400 g/mol, 13,500 g/mol, 13,600 g/mol, 13,700 g/mol, 13,800 g/mol, 13,900 g/mol, 14,000 g/mol, 14,100 g/mol, 14,200 g/mol, 14,300 g/mol, 14,400 g/mol, 14,500 g/mol, 14,600 g/mol, 14,700 g/mol, 14,800 g/mol, 14,900 g/mol, or 15,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of from about 10,000 g/mol to about 15,000 g/mol (e.g., about 10,000 g/mol, 10,100 g/mol, 10,200 g/mol, 10,300 g/mol, 10,400 g/mol, 10,500 g/mol, 10,600 g/mol, 10,700 g/mol, 10,800 g/mol, 10,900 g/mol, 11,000 g/mol, 11,100 g/mol, 11,200 g/mol, 11,300 g/mol, 11,400 g/mol, 11,500 g/mol, 11,600 g/mol, 11,700 g/mol, 11,800 g/mol, 11,900 g/mol, 12,000 g/mol, 12,100 g/mol, 12,200 g/mol, 12,300 g/mol, 12,400 g/mol, 12,500 g/mol, 12,600 g/mol, 12,700 g/mol, 12,800 g/mol, 12,900 g/mol, 13,000 g/mol, 13,100 g/mol, 13,200 g/mol, 13,300 g/mol, 13,400 g/mol, 13,500 g/mol, 13,600 g/mol, 13,700 g/mol, 13,800 g/mol, 13,900 g/mol, 14,000 g/mol, 14,100 g/mol, 14,200 g/mol, 14,300 g/mol, 14,400 g/mol, 14,500 g/mol, 14,600 g/mol, 14,700 g/mol, 14,800 g/mol, 14,900 g/mol, or 15,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of from about 11,500 g/mol to about 15,000 g/mol (e.g., about 11,500 g/mol, 11,600 g/mol, 11,700 g/mol, 11,800 g/mol, 11,900 g/mol, 12,000 g/mol, 12,100 g/mol, 12,200 g/mol, 12,300 g/mol, 12,400 g/mol, 12,500 g/mol, 12,600 g/mol, 12,700 g/mol, 12,800 g/mol, 12,900 g/mol, 13,000 g/mol, 13,100 g/mol, 13,200 g/mol, 13,300 g/mol, 13,400 g/mol, 13,500 g/mol, 13,600 g/mol, 13,700 g/mol, 13,800 g/mol, 13,900 g/mol, 14,000 g/mol, 14,100 g/mol, 14,200 g/mol, 14,300 g/mol, 14,400 g/mol, 14,500 g/mol, 14,600 g/mol, 14,700 g/mol, 14,800 g/mol, 14,900 g/mol, or 15,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of from about 12,000 g/mol to about 15,000 g/mol (e.g., about 12,000 g/mol, 12,100 g/mol, 12,200 g/mol, 12,300 g/mol, 12,400 g/mol, 12,500 g/mol, 12,600 g/mol, 12,700 g/mol, 12,800 g/mol, 12,900 g/mol, 13,000 g/mol, 13,100 g/mol, 13,200 g/mol, 13,300 g/mol, 13,400 g/mol, 13,500 g/mol, 13,600 g/mol, 13,700 g/mol, 13,800 g/mol, 13,900 g/mol, 14,000 g/mol, 14,100 g/mol, 14,200 g/mol, 14,300 g/mol, 14,400 g/mol, 14,500 g/mol, 14,600 g/mol, 14,700 g/mol, 14,800 g/mol, 14,900 g/mol, or 15,000 g/mol).

In some embodiments, the poloxamer has an average molar mass of from about 12,500 g/mol to about 15,000 g/mol (e.g., about 12,500 g/mol, 12,600 g/mol, 12,700 g/mol, 12,800 g/mol, 12,900 g/mol, 13,000 g/mol, 13,100 g/mol, 13,200 g/mol, 13,300 g/mol, 13,400 g/mol, 13,500 g/mol, 13,600 g/mol, 13,700 g/mol, 13,800 g/mol, 13,900 g/mol, 14,000 g/mol, 14,100 g/mol, 14,200 g/mol, 14,300 g/mol, 14,400 g/mol, 14,500 g/mol, 14,600 g/mol, 14,700 g/mol, 14,800 g/mol, 14,900 g/mol, or 15,000 g/mol).

Poloxamers P288, P335, P338, and P407

Poloxamers that may be used in conjunction with the compositions and methods of the disclosure include “poloxamer 288” (also referred to in the art as “P 288” and poloxamer “F98”) having the approximate chemical formula $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$, wherein the sum of x and y is about 236.36, and z is about 44.83. The average molecular weight of P288 is about 13,000 g/mol.

In some embodiments, the poloxamer is a variant of P288, such as a variant of the formula $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$, wherein the sum of x and y is from about 220 to about 250, and z is from about 40 to about 50. In some embodiments, the average molecular weight of the poloxamer is from about 12,000 g/mol to about 14,000 g/mol.

Poloxamers that may be used in conjunction with the compositions and methods of the disclosure further include “poloxamer 335” (also referred to in the art as “P 335” and poloxamer “P105”), having the approximate chemical formula $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$, wherein the sum of x and y is about 73.86, and z is about 56.03. The average molecular weight of P335 is about 6,500 g/mol.

In some embodiments, the poloxamer is a variant of P335, such as a variant of the formula $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$, wherein the sum of x and y is from about 60 to about 80, and z is from about 50 to about 60. In some embodiments, the average molecular weight of the poloxamer is from about 6,000 g/mol to about 7,000 g/mol.

Poloxamers that may be used in conjunction with the compositions and methods of the disclosure further include “poloxamer 338” (also referred to in the art as “P 338” and poloxamer “F108”), having the approximate chemical formula $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$, wherein the sum of x and y is about 265.45, and z is about 50.34. The average molecular weight of P335 is about 14,600 g/mol.

In some embodiments, the poloxamer is a variant of P338, such as a variant of the formula $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$, wherein the sum of x and y is from about 260 to about 270, and z is from about 45 to about 55. In some embodiments, the average molecular weight of the poloxamer is from about 14,000 g/mol to about 15,000 g/mol.

Poloxamers that may be used in conjunction with the compositions and methods of the disclosure further include “poloxamer 407” (also referred to in the art as “P 407” and poloxamer “F127”), having the approximate chemical formula $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$, wherein the sum of x and y is about 200.45, and z is about 65.17. The average molecular weight of P335 is about 12,600 g/mol.

In some embodiments, the poloxamer is a variant of P407, such as a variant of the formula $\text{HO}(\text{C}_2\text{H}_4\text{O})_x(\text{C}_3\text{H}_6\text{O})_y(\text{C}_2\text{H}_4\text{O})_z\text{H}$, wherein the sum of x and y is from about 190 to about 210, and z is from about 60 to about 70. In some embodiments, the average molecular weight of the poloxamer is from about 12,000 g/mol to about 13,000 g/mol.

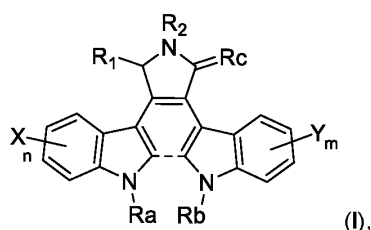
For clarity, the terms “average molar mass” and “average molecular weight” are used interchangeable herein to refer to the same quantity. The average molar mass, ethylene oxide content, and propylene oxide content of a poloxamer, as described herein, can be determined using methods disclosed in Alexandridis and Hatton, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 96:1-46 (1995), the disclosure of which is incorporated herein by reference in its entirety.

Transduction using a protein kinase C modulator

A variety of agents can be used to reduce PKC activity and/or expression during viral transduction. Without being limited by mechanism, such agents can augment viral transduction by stimulating Akt signal transduction and/or maintaining cofilin in a dephosphorylated state, thereby promoting actin depolymerization. This actin depolymerization event may serve to remove a physical barrier that hinders entry of a viral vector into the nucleus of a target cell.

Staurosporine and variants thereof

In some embodiments, the substance that reduces activity and/or expression of PKC is a PKC inhibitor. The PKC inhibitor may be staurosporine or a variant thereof. For example, the PKC inhibitor may be a compound represented by formula (I)



wherein R_1 is H, OH, optionally substituted alkoxy, optionally substituted acyloxy, optionally substituted amino, optionally substituted alkylamino, optionally substituted amido, halogen, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted C_{2-6} alkynyl, optionally substituted acyl, optionally substituted alkoxy carbonyl, oxo, thiocarbonyl, optionally substituted carboxy, or ureido;

R_2 is H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted C_{2-6} alkynyl, or optionally substituted acyl;

R_a and R_b are each, independently, H, optionally substituted C₁₋₆ alkyl, optionally substituted C₂₋₆ alkenyl, or optionally substituted C₂₋₆ alkynyl, optionally substituted and optionally fused aryl, optionally substituted and optionally fused heteroaryl, optionally substituted and optionally fused cycloalkyl, or optionally substituted and optionally fused heterocycloalkyl, or R_a and R_b, together with
5 the atoms to which they are bound, are joined to form an optionally substituted and optionally fused heterocycloalkyl ring;

R_c is O, NR_d, or S;

R_d is H, optionally substituted C₁₋₆ alkyl, optionally substituted C₂₋₆ alkenyl, or optionally substituted C₂₋₆ alkynyl;

10 each X is, independently, halogen, optionally substituted haloalkyl, cyano, optionally substituted amino, hydroxyl, thiol, optionally substituted alkoxy, optionally substituted alkylthio, optionally substituted acyloxy, optionally substituted alkoxycarbonyl, optionally substituted carboxy, ureido, optionally substituted alkyl sulfonyl, optionally substituted aryl sulfonyl, optionally substituted heteroaryl sulfonyl, optionally substituted cycloalkyl sulfonyl, optionally substituted heterocycloalkyl
15 sulfonyl, optionally substituted alkyl sulfanyl, optionally substituted aryl sulfanyl, optionally substituted heteroaryl sulfanyl, optionally substituted cycloalkyl sulfanyl, optionally substituted heterocycloalkyl sulfanyl, optionally substituted alkyl sulfinyl, optionally substituted aryl sulfinyl, optionally substituted heteroaryl sulfinyl, optionally substituted cycloalkyl sulfinyl, optionally substituted heterocycloalkyl sulfinyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl,
20 optionally substituted and optionally fused aryl, optionally substituted and optionally fused heteroaryl, optionally substituted and optionally fused cycloalkyl, or optionally substituted and optionally fused heterocycloalkyl;

each Y is, independently, halogen, optionally substituted haloalkyl, cyano, optionally substituted amino, hydroxyl, thiol, optionally substituted alkoxy, optionally substituted alkylthio,
25 optionally substituted acyloxy, optionally substituted alkoxycarbonyl, optionally substituted carboxy, ureido, optionally substituted alkyl sulfonyl, optionally substituted aryl sulfonyl, optionally substituted heteroaryl sulfonyl, optionally substituted cycloalkyl sulfonyl, optionally substituted heterocycloalkyl sulfonyl, optionally substituted alkyl sulfanyl, optionally substituted aryl sulfanyl, optionally substituted heteroaryl sulfanyl, optionally substituted cycloalkyl sulfanyl, optionally substituted heterocycloalkyl
30 sulfanyl, optionally substituted alkyl sulfinyl, optionally substituted aryl sulfinyl, optionally substituted heteroaryl sulfinyl, optionally substituted cycloalkyl sulfinyl, optionally substituted heterocycloalkyl sulfinyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted and optionally fused aryl, optionally substituted and optionally fused heteroaryl, optionally substituted and optionally fused cycloalkyl, or optionally substituted and optionally fused
35 heterocycloalkyl;

--- represents a bond that is optionally present;

n is an integer from 0-4; and

m is an integer from 0-4;

or a salt thereof.

Interfering RNA

Exemplary PKC modulating agents that may be used in conjunction with the compositions and methods of the disclosure include interfering RNA molecules, such as short interfering RNA (siRNA), short hairpin RNA (shRNA), and/or micro RNA (miRNA), that diminish PKC gene expression. Methods for producing interfering RNA molecules are known in the art and are described in detail, for example, in WO 2004/044136 and US Patent No. 9,150,605, the disclosures of each of which are incorporated herein by reference in their entirety.

10 *Transduction using a cyclosporine*

In some embodiments, therapeutic cells of the disclosure are produced by transducing the cells in the presence of a cyclosporine, such as cyclosporine A (CsA) or cyclosporine H (CsH).

In some embodiments, the concentration of the cyclosporine, when contacted with the cell, is from about 1 μ M to about 10 μ M (e.g., about 1 μ M, 1.1 μ M, 1.2 μ M, 1.3 μ M, 1.4 μ M, 1.5 μ M, 1.6 μ M, 1.7 μ M, 1.8 μ M, 1.9 μ M, 2 μ M, 2.1 μ M, 2.2 μ M, 2.3 μ M, 2.4 μ M, 2.5 μ M, 2.6 μ M, 2.7 μ M, 2.8 μ M, 2.9 μ M, 3 μ M, 3.1 μ M, 3.2 μ M, 3.3 μ M, 3.4 μ M, 3.5 μ M, 3.6 μ M, 3.7 μ M, 3.8 μ M, 3.9 μ M, 4 μ M, 4.1 μ M, 4.2 μ M, 4.3 μ M, 4.4 μ M, 4.5 μ M, 4.6 μ M, 4.7 μ M, 4.8 μ M, 4.9 μ M, 5 μ M, 5.1 μ M, 5.2 μ M, 5.3 μ M, 5.4 μ M, 5.5 μ M, 5.6 μ M, 5.7 μ M, 5.8 μ M, 5.9 μ M, 6 μ M, 6.1 μ M, 6.2 μ M, 6.3 μ M, 6.4 μ M, 6.5 μ M, 6.6 μ M, 6.7 μ M, 6.8 μ M, 6.9 μ M, 7 μ M, 7.1 μ M, 7.2 μ M, 7.3 μ M, 7.4 μ M, 7.5 μ M, 7.6 μ M, 7.7 μ M, 7.8 μ M, 7.9 μ M, 8 μ M, 8.1 μ M, 8.2 μ M, 8.3 μ M, 8.4 μ M, 8.5 μ M, 8.6 μ M, 8.7 μ M, 8.8 μ M, 8.9 μ M, 9 μ M, 9.1 μ M, 9.2 μ M, 9.3 μ M, 9.4 μ M, 9.5 μ M, 9.6 μ M, 9.7 μ M, 9.8 μ M, 9.9 μ M, or 10 μ M).

Transduction using an activator of prostaglandin E receptor signaling

In some embodiments, therapeutic cells of the disclosure are produced by transducing the cells in the presence of an activator of prostaglandin E receptor signaling.

In some embodiments, the activator of prostaglandin E receptor signaling is a small molecule, such as a compound described in WO 2007/112084 or WO 2010/108028, the disclosures of each of which are incorporated herein by reference as they pertain to prostaglandin E receptor signaling activators.

In some embodiments, the activator of prostaglandin E receptor signaling is a small molecule, such as a small organic molecule, a prostaglandin, a Wnt pathway agonist, a cAMP/PI3K/AKT pathway agonist, a Ca^{2+} second messenger pathway agonist, a nitric oxide (NO)/angiotensin signaling agonist, or another compound known to stimulate the prostaglandin signaling pathway, such as a compound selected from Mebeverine, Flurandrenolide, Atenolol, Pindolol, Gaboxadol, Kynurenic Acid, Hydralazine, Thiabendazole, Bicuclline, Vesamicol, Peruvsoside, Imipramine, Chlorpropamide, 1,5-Pentamethylenetetrazole, 4-Aminopyridine, Diazoxide, Benfotiamine, 12-Methoxydodecenoic acid, N-Formyl-Met-Leu-Phe, Gallamine, IAA 94, Chlorotrianisene, and or a derivative of any of these compounds.

In some embodiments, the activator of prostaglandin E receptor signaling is a naturally occurring or synthetic chemical molecule or polypeptide that binds to and/or interacts with a

prostaglandin E receptor, typically to activate or increase one or more of the downstream signaling pathways associated with a prostaglandin E receptor.

In some embodiments, the activator of prostaglandin E receptor signaling is selected from the group consisting of prostaglandin (PG) A₂ (PGA₂), PGB₂, PGD₂, PGE₁ (Alprostadil), PGE₂, PGF₂,
 5 PGI₂ (Epoprostenol), PGH₂, PGJ₂, and derivatives and analogs thereof.

In some embodiments, the activator of prostaglandin E receptor signaling is PGE₂ or dmPGE₂.

In some embodiments, the activator of prostaglandin E receptor signaling is 15d-PGJ₂,
 deltaI₂-PGJ₂, 2-hydroxyheptadecatrienoic acid (HHT), Thromboxane (TXA₂ and TXB₂), PGI₂
 10 analogs, e.g., Iloprost and Treprostinil, PGF₂ analogs, e.g., Travoprost, Carboprost tromethamine, Tafluprost, Latanoprost, Bimatoprost, Unoprostone isopropyl, Cloprostenol, Oestrophane, and Superphane, PGE₁ analogs, e.g., 11-deoxy PGE₁, Misoprostol, and Butaprost, and Corey alcohol-A ([3aa,4a,5,6aa]-(-)-[Hexahydro-4-(hydroxymethyl)-2-oxo-2H-cyclopenta[b]furan-5-yl][1,1'-biphenyl]-4-carboxylate), Corey alcohol-B (2H-Cyclopenta[b]furan-2-one,5-(benzoyloxy)hexahydro-4-
 15 (hydroxymethyl)[3aR-(3aa,4a,5,6aa)]), and Corey diol ((3aR,4S,5R,6aS)-hexahydro-5-hydroxy-4-(hydroxymethyl)-2H-cyclopenta[b]furan-2-one).

In some embodiments, the activator of prostaglandin E receptor signaling is a prostaglandin E receptor ligand, such as prostaglandin E₂ (PGE₂), or an analog or derivative thereof. Prostaglandins refer generally to hormone-like molecules that are derived from fatty acids containing 20 carbon
 20 atoms, including a 5-carbon ring, as described herein and known in the art. Illustrative examples of PGE₂ "analogs" or "derivatives" include, but are not limited to, 16,16-dimethyl PGE₂, 16,16 dimethyl PGE₂ p-(p-acetamidobenzamido) phenyl ester, 11-deoxy-16,16-dimethyl PGE₂, 9-deoxy-9-methylene-16, 16-dimethyl PGE₂, 9-deoxy-9-methylene PGE₂, 9-keto Fluprostenol, 5-trans PGE₂, 17-phenyl-omega-trinor PGE₂, PGE₂ serinol amide, PGE₂ methyl ester, 16-phenyl tetranor PGE₂, 15(S)- 15-methyl PGE₂, 15 (R)- 15-methyl PGE₂, 8-iso-15-keto PGE₂, 8-iso PGE₂ isopropyl ester, 20-hydroxy
 25 PGE₂, nocolprost, sulprostone, butaprost, 15-keto PGE₂, and 19 (R) hydroxy PGE₂.

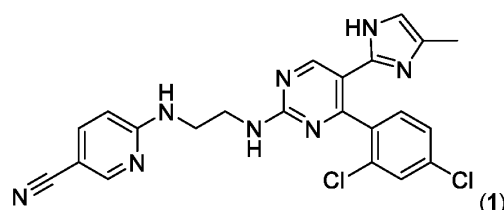
In some embodiments, the activator of prostaglandin E receptor signaling is a prostaglandin analog or derivative having a similar structure to PGE₂ that is substituted with halogen at the 9-position (see, e.g., WO 2001/12596, herein incorporated by reference in its entirety), as well as 2-decarboxy-2-phosphinico prostaglandin derivatives, such as those described in US 2006/0247214,
 30 herein incorporated by reference in its entirety).

In some embodiments, the activator of prostaglandin E receptor signaling is a non-PGE₂-based ligand. In some embodiments, the activator of prostaglandin E receptor signaling is CAY10399, ONO_8815Ly, ONO-AE1-259, or CP-533,536. Additional examples of non-PGE₂-based
 35 EP₂ agonists include the carbazoles and fluorenes disclosed in WO 2007/071456, herein incorporated by reference for its disclosure of such agents. Illustrative examples of non-PGE₂-based EP₃ agonist include, but are not limited to, AE5-599, MB28767, GR 63799X, ONO- NT012, and ONO-AE-248. Illustrative examples of non-PGE₂-based EP₄ agonist include, but are not limited to, ONO-4819, APS-999 Na, AH23848, and ONO-AE 1- 329. Additional examples of non-PGE₂-based EP₄

agonists can be found in WO 2000/038663; US Patent No. 6,747,037; and US Patent No. 6,610,719, each of which are incorporated by reference for their disclosure of such agonists

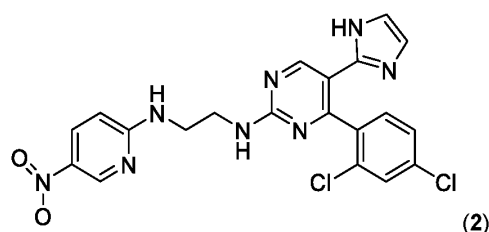
In some embodiments, the activator of prostaglandin E receptor signaling is a Wnt agonist. Illustrative examples of Wnt agonists include, but are not limited to, Wnt polypeptides and glycogen synthase kinase 3 (GSK3) inhibitors. Illustrative examples of Wnt polypeptides suitable for use as compounds that stimulate the prostaglandin EP receptor signaling pathway include, but are not limited to, Wnt1, Wnt2, Wnt2b/13, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt7c, Wnt8, Wnt8a, Wnt8b, Wnt8c, Wnt10a, Wnt10b, Wnt11, Wnt14, Wnt15, or biologically active fragments thereof. GSK3 inhibitors suitable for use as agents that stimulate the prostaglandin EP receptor signaling pathway bind to and decrease the activity of GSK3a, or GSK3. Illustrative examples of GSK3 inhibitors include, but are not limited to, BIO (6-bromoindirubin-3'-oxime), LiCl, Li₂CO₃, or other GSK-3 inhibitors, as exemplified in US Patents Nos. 6,057,117 and 6,608,063, as well as US 2004/0092535 and US 2004/0209878, and ATP-competitive, selective GSK-3 inhibitors CHIR-911 and CHIR-837 (also referred to as CT-99021/CHIR-99021 and CT-98023/CHIR-98023, respectively) (Chiron Corporation (Emeryville, CA)).

The structure of CHIR-99021 is



or a salt thereof.

The structure of CHIR-98023 is



or a salt thereof.

In some embodiments, method further includes contacting the cell with a GSK3 inhibitor.

In some embodiments, the GSK3 inhibitor is CHIR-99021 or CHIR-98023.

In some embodiments, the GSK3 inhibitor is Li₂CO₃.

In some embodiments, the activator of prostaglandin E receptor signaling is an agent that increases signaling through the cAMP/P13K/AKT second messenger pathway, such as an agent selected from the group consisting of dibutyryl cAMP (DBcAMP), phorbol ester, forskolin, sclareline, 8-bromo-cAMP, cholera toxin (CTx), aminophylline, 2,4 dinitrophenol (DNP), norepinephrine, epinephrine, isoproterenol, isobutylmethylxanthine (IBMX), caffeine, theophylline (dimethylxanthine), dopamine, rolipram, iloprost, pituitary adenylate cyclase activating polypeptide (PACAP), and vasoactive intestinal polypeptide (VIP), and derivatives of these agents.

In some embodiments, the activator of prostaglandin E receptor signaling is an agent that increases signaling through the Ca^{2+} second messenger pathway, such as an agent selected from the group consisting of Bapta-AM, Fendiline, Nicardipine, and derivatives of these agents.

5 In some embodiments, the activator of prostaglandin E receptor signaling is an agent that increases signaling through the NO/ Angiotensin signaling, such as an agent selected from the group consisting of L-Arg, Sodium Nitroprusside, Sodium Vanadate, Bradykinin, and derivatives thereof.

Transduction using a polycationic polymer

10 In some embodiments, therapeutic cells of the disclosure are produced by transducing the cells in the presence of a polycationic polymer. In some embodiments, the polycationic polymer is polybrene, protamine sulfate, polyethylenimine, or a polyethylene glycol/poly-L-lysine block copolymer.

In some embodiments, the polycationic polymer is protamine sulfate.

15 In some embodiments, the cell is further contacted with an expansion agent during the transduction procedure. The cell may be, for example, a hematopoietic stem cell and the expansion agent may be a hematopoietic stem cell expansion agent, such as a hematopoietic stem cell expansion agent known in the art or described herein.

Transduction using an HDAC inhibitor

20 A variety of agents can be used to inhibit histone deacetylases in order to increase the expression of a transgene during viral transduction. Without wishing to be bound by theory, reduced transgene expression from viral vectors may be caused by epigenetic silencing of vector genomes carried out by histone deacetylases. Hydroxamic acids represent a particularly robust class of HDAC inhibitors that inhibit these enzymes by virtue of hydroxamate functionality that binds cationic zinc within the active sites of these enzymes. Exemplary inhibitors include trichostatin A, as well as Vorinostat (N-hydroxy-N'-phenyl-octanediamide, described in Marks et al., Nature Biotechnology 25, 84 to 90 (2007); Stenger, Community Oncology 4, 384-386 (2007), the disclosures of which are incorporated by reference herein). Other HDAC inhibitors include Panobinostat, described in Drugs of the Future 32(4): 315-322 (2007), the disclosure of which is incorporated herein by reference.

30 Additional examples of hydroxamic acid inhibitors of histone deacetylases include the compounds shown below, described in Bertrand, European Journal of Medicinal Chemistry 45:2095-2116 (2010), the disclosure of which is incorporated herein by reference.

Other HDAC inhibitors that do not contain a hydroxamate substituent have also been developed, including Valproic acid (Gottlicher, et al., EMBO J. 20(24): 6969-6978 (2001) and Mocetinostat (N-(2-Aminophenyl)-4-[[[4-(pyridin-3-yl)pyrimidin-2-yl]amino]methyl]benzamide, described in Balasubramanian et al., Cancer Letters 280: 211-221 (2009)), the disclosure of each of which is incorporated herein by reference. Other small molecule inhibitors that exploit chemical functionality distinct from a hydroxamate include those described in Bertrand, European Journal of Medicinal Chemistry 45:2095-2116 (2010), the disclosure of which is incorporated herein by reference.

Additional examples of chemical modulators of histone acetylation useful with the compositions and methods of the invention include modulators of HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, Sirt1, Sirt2, and/or HAT, such as butyrylhydroxamic acid, M344, LAQ824 (Dacinostat), AR-42, Belinostat (PXD101), CUDC-101, Scriptaid, Sodium Phenylbutyrate, Tasquinimod, Quisinostat (JNJ-26481585), Pracinostat (SB939), CUDC-907, Entinostat (MS-275), Mocetinostat (MGCD0103), Tubastatin A HCl, PCI-34051, Droxinostat, PCI-24781 (Abexinostat), RGFP966, Rocilinostat (ACY-1215), CI994 (Tacedinaline), Tubacin, RG2833 (RGFP109), Resminostat, Tubastatin A, BRD73954, BG45, 4SC-202, CAY10603, LMK-235, Nexturastat A, TMP269, HPOB, Cambinol, and Anacardic Acid.

In some particular embodiments, the HDAC inhibitor is Scriptaid.

Additional transduction enhancers

In some embodiments of the methods described herein, during the transduction procedure, the cell is further contacted with an agent that inhibits mTOR signaling. The agent that inhibits mTOR signaling may be, for example, rapamycin, among other suppressors of mTOR signaling.

Additional transduction enhancers that may be used in conjunction with the compositions and methods of the disclosure include, for example, tacrolimus and vectorfusin.

Spinoculation

In some embodiments of the disclosure, a cell targeted for transduction may be spun e.g., by centrifugation, while being cultured with a viral vector (e.g., in combination with one or more additional agents described herein). This "spinoculation" process may occur with a centripetal force of, e.g., from about 200 x g to about 2,000 x g. The centripetal force may be, e.g., from about 300 x g to about 1,200 x g (e.g., about 300 x g, 400 x g, 500 x g, 600 x g, 700 x g, 800 x g, 900 x g, 1,000 x g, 1,100 x g, or 1,200 x g, or more). In some embodiments, the cell is spun for from about 10 minutes to about 3 hours (e.g., about 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 60 minutes, 65 minutes, 70 minutes, 75 minutes, 80 minutes, 85 minutes, 90 minutes, 95 minutes, 100 minutes, 105 minutes, 110 minutes, 115 minutes, 120 minutes, 125 minutes, 130 minutes, 135 minutes, 140 minutes, 145 minutes, 150 minutes, 155 minutes, 160 minutes, 165 minutes, 170 minutes, 175 minutes, 180 minutes, or more). In some embodiments, the cell is spun at room temperature, such as at a temperature of about 25° C.

Exemplary transduction protocols involving a spinoculation step are described, e.g., in Millington et al., PLoS One 4:e6461 (2009); Guo et al., Journal of Virology 85:9824-9833 (2011); O'Doherty et al., Journal of Virology 74:10074-10080 (2000); and Federico et al., Lentiviral Vectors and Exosomes as Gene and Protein Delivery Tools, Methods in Molecular Biology 1448, Chapter 4 (2016), the disclosures of each of which are incorporated herein by reference.

Viral Vectors for C1-INH Expression

Viral genomes provide a rich source of vectors that can be used for the efficient delivery of exogenous genes into a mammalian cell. Viral genomes are particularly useful vectors for gene

delivery as the polynucleotides contained within such genomes are typically incorporated into the nuclear genome of a mammalian cell by generalized or specialized transduction. These processes occur as part of the natural viral replication cycle, and do not require added proteins or reagents in order to induce gene integration. Examples of viral vectors are a retrovirus (e.g., Retroviridae family viral vector), adenovirus (e.g., Ad5, Ad26, Ad34, Ad35, and Ad48), parvovirus, coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g. measles and Sendai), positive strand RNA viruses, such as picornavirus and alphavirus, and double stranded DNA viruses including adenovirus, herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, modified vaccinia Ankara (MVA), fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, human papilloma virus, human foamy virus, and hepatitis virus, for example. Examples of retroviruses are: avian leukemia-sarcoma, avian C-type viruses, mammalian C-type, B-type viruses, D-type viruses, oncoretroviruses, HTLV-BLV group, lentivirus, alpharetrovirus, gammaretrovirus, spumavirus (Coffin, J. M., Retroviridae: The viruses and their replication, Virology, Third Edition (Lippincott-Raven, Philadelphia, (1996))). Other examples are murine leukemia viruses, murine sarcoma viruses, mouse mammary tumor virus, bovine leukemia virus, feline leukemia virus, feline sarcoma virus, avian leukemia virus, human T-cell leukemia virus, baboon endogenous virus, Gibbon ape leukemia virus, Mason Pfizer monkey virus, simian immunodeficiency virus, simian sarcoma virus, Rous sarcoma virus and lentiviruses. Other examples of vectors are described, for example, in McVey et al., (US 5,801,030), the teachings of which are incorporated herein by reference.

Retroviral vectors

The delivery vector used in the methods and compositions described herein may be a retroviral vector. One type of retroviral vector that may be used in the methods and compositions described herein is a lentiviral vector. Lentiviral vectors (LVs), a subset of retroviruses, transduce a wide range of dividing and non-dividing cell types with high efficiency, conferring stable, long-term expression of the transgene. An overview of optimization strategies for packaging and transducing LVs is provided in Delenda, The Journal of Gene Medicine 6: S125 (2004), the disclosure of which is incorporated herein by reference.

The use of lentivirus-based gene transfer techniques relies on the in vitro production of recombinant lentiviral particles carrying a highly deleted viral genome in which the transgene of interest is accommodated. In particular, the recombinant lentivirus are recovered through the in trans coexpression in a permissive cell line of (1) the packaging constructs, i.e., a vector expressing the Gag-Pol precursors together with Rev (alternatively expressed in trans); (2) a vector expressing an envelope receptor, generally of an heterologous nature; and (3) the transfer vector, consisting in the viral cDNA deprived of all open reading frames, but maintaining the sequences required for replication, encapsidation, and expression, in which the sequences to be expressed are inserted.

A LV used in the methods and compositions described herein may include one or more of a 5'-Long terminal repeat (LTR), HIV signal sequence, HIV Psi signal 5'-splice site (SD), delta-GAG

element, Rev Responsive Element (RRE), 3'-splice site (SA), elongation factor (EF) 1-alpha promoter and 3'-self inactivating LTR (SIN-LTR). The lentiviral vector optionally includes a central polypurine tract (cPPT) and a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), as described in US 6,136,597, the disclosure of which is incorporated herein by reference as it pertains to WPRE. The lentiviral vector may further include a pHR' backbone, which may include for example as provided below.

The Lentigen LV described in Lu et al., Journal of Gene Medicine 6:963 (2004) may be used to express the DNA molecules and/or transduce cells. A LV used in the methods and compositions described herein may a 5'-Long terminal repeat (LTR), HIV signal sequence, HIV Psi signal 5'-splice site (SD), delta-GAG element, Rev Responsive Element (RRE), 3'-splice site (SA), elongation factor (EF) 1-alpha promoter and 3'-self inactivating L TR (SIN-LTR). It will be readily apparent to one skilled in the art that optionally one or more of these regions is substituted with another region performing a similar function.

Enhancer elements can be used to increase expression of modified DNA molecules or increase the lentiviral integration efficiency. The LV used in the methods and compositions described herein may include a nef sequence. The LV used in the methods and compositions described herein may include a cPPT sequence which enhances vector integration. The cPPT acts as a second origin of the (+)-strand DNA synthesis and introduces a partial strand overlap in the middle of its native HIV genome. The introduction of the cPPT sequence in the transfer vector backbone strongly increased the nuclear transport and the total amount of genome integrated into the DNA of target cells. The LV used in the methods and compositions described herein may include a Woodchuck Posttranscriptional Regulatory Element (WPRE). The WPRE acts at the transcriptional level, by promoting nuclear export of transcripts and/or by increasing the efficiency of polyadenylation of the nascent transcript, thus increasing the total amount of mRNA in the cells. The addition of the WPRE to LV results in a substantial improvement in the level of transgene expression from several different promoters, both in vitro and in vivo. The LV used in the methods and compositions described herein may include both a cPPT sequence and WPRE sequence. The vector may also include an IRES sequence that permits the expression of multiple polypeptides from a single promoter.

In addition to IRES sequences, other elements which permit expression of multiple polypeptides are useful. The vector used in the methods and compositions described herein may include multiple promoters that permit expression more than one polypeptide. The vector used in the methods and compositions described herein may include a protein cleavage site that allows expression of more than one polypeptide. Examples of protein cleavage sites that allow expression of more than one polypeptide are described in Klump et al., Gene Ther.; 8:811 (2001), Osborn et al., Molecular Therapy 12:569 (2005), Szymczak and Vignali, Expert Opin Biol Ther. 5:627 (2005), and Szymczak et al., Nat Biotechnol. 22:589 (2004), the disclosures of which are incorporated herein by reference as they pertain to protein cleavage sites that allow expression of more than one polypeptide. It will be readily apparent to one skilled in the art that other elements that permit expression of multiple polypeptides identified in the future are useful and may be utilized in the vectors suitable for use with the compositions and methods described herein.

The vector used in the methods and compositions described herein may, be a clinical grade vector.

The viral vectors (e.g., retroviral vectors, e.g., lentiviral vectors) may include a promoter operably coupled to the transgene to control gene expression. The promoter may be a ubiquitous promoter. Alternatively, the promoter may be a tissue specific promoter, such as a myeloid cell-specific or hepatocyte-specific promoter. Suitable promoters that may be used with the compositions described herein include CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, elongation factor 1 α (EF1 α) promoter, EF1 α short form (EFS) promoter, phosphoglycerate kinase (PGK) promoter, α -globin promoter, and β -globin promoter. In some embodiments, the promoter is a C1-INH promoter, e.g., as described in Zahedi et al. *Inflammation*, 26:183-191, 2002, and Zahedi et al. *J Immunol* 162:7249-7255 1999, the disclosures of which are hereby incorporated in their entirety. Other promoters that may be used include, e.g., DC172 promoter, human serum albumin promoter, alpha1 antitrypsin promoter, thyroxine binding globulin promoter. The DC172 promoter is described in Jacob, et al. *Gene Ther.* 15:594-603, 2008, hereby incorporated by reference in its entirety.

The viral vectors (e.g., retroviral vectors, e.g., lentiviral vectors) may include an enhancer operably coupled to the transgene to control gene expression. The enhancer may include a β -globin locus control region (β LCR).

In some embodiments, the viral vector further includes a miRNA targeting sequence, e.g., operably linked to the transgene. For example, the miRNA targeting sequence may have complementarity to a miRNA that is endogenously expressed in a tissue in which expression of C1-INH is undesirable. A miRNA targeting sequence may be used to suppress expression in undesirable cell types.

25 **Methods of Producing Functional C1-INH-Expressing Cells by Ex Vivo Transfection**

One platform that can be used to achieve therapeutically effective intracellular concentrations of one or more proteins described herein in mammalian cells is via the stable expression of genes encoding these agents (e.g., by integration into the nuclear or mitochondrial genome of a mammalian cell). These genes are polynucleotides that encode the primary amino acid sequence of the corresponding protein. In order to introduce such exogenous genes into a mammalian cell, these genes can be incorporated into a vector. Vectors can be introduced into a cell by a variety of methods, including transformation, transfection, direct uptake, projectile bombardment, and by encapsulation of the vector in a liposome. Examples of suitable methods of transfecting or transforming cells are calcium phosphate precipitation, electroporation, microinjection, infection, lipofection, and direct uptake. Such methods are described in more detail, for example, in Green et al., *Molecular Cloning: A Laboratory Manual*, Fourth Edition (Cold Spring Harbor University Press, New York (2014)); and Ausubel et al., *Current Protocols in Molecular Biology* (John Wiley & Sons, New York (2015)), the disclosures of each of which are incorporated herein by reference.

Genes encoding therapeutic proteins of the disclosure can also be introduced into mammalian cells by targeting a vector containing a gene encoding such an agent to cell membrane

phospholipids. For example, vectors can be targeted to the phospholipids on the extracellular surface of the cell membrane by linking the vector molecule to a VSV-G protein, a viral protein with affinity for all cell membrane phospholipids. Such, a construct can be produced using methods well known to those of skill in the field.

5 Recognition and binding of the polynucleotide encoding one or more therapeutic proteins of the disclosure by mammalian RNA polymerase is important for gene expression. As such, one may include sequence elements within the polynucleotide that exhibit a high affinity for transcription factors that recruit RNA polymerase and promote the assembly of the transcription complex at the transcription initiation site. Such sequence elements include, e.g., a mammalian promoter, the
10 sequence of which can be recognized and bound by specific transcription initiation factors and ultimately RNA polymerase. Examples of mammalian promoters have been described in Smith et al., *Mol. Sys. Biol.*, 3:73, online publication, the disclosure of which is incorporated herein by reference.

 Once a polynucleotide encoding one or more therapeutic proteins has been incorporated into the nuclear DNA of a mammalian cell, transcription of this polynucleotide can be induced by methods
15 known in the art. For example, expression can be induced by exposing the mammalian cell to an external chemical reagent, such as an agent that modulates the binding of a transcription factor and/or RNA polymerase to the mammalian promoter and thus regulates gene expression. The chemical reagent can serve to facilitate the binding of RNA polymerase and/or transcription factors to the mammalian promoter, e.g., by removing a repressor protein that has bound the promoter.
20 Alternatively, the chemical reagent can serve to enhance the affinity of the mammalian promoter for RNA polymerase and/or transcription factors such that the rate of transcription of the gene located downstream of the promoter is increased in the presence of the chemical reagent. Examples of chemical reagents that potentiate polynucleotide transcription by the above mechanisms are tetracycline and doxycycline. These reagents are commercially available (Life Technologies,
25 Carlsbad, CA) and can be administered to a mammalian cell in order to promote gene expression according to established protocols.

 Other DNA sequence elements that may be included in polynucleotides for use in the compositions and methods described herein are enhancer sequences. Enhancers represent another class of regulatory elements that induce a conformational change in the polynucleotide containing the
30 gene of interest such that the DNA adopts a three-dimensional orientation that is favorable for binding of transcription factors and RNA polymerase at the transcription initiation site. Thus, polynucleotides for use in the compositions and methods described herein include those that encode one or more therapeutic proteins and additionally include a mammalian enhancer sequence. Many enhancer sequences are now known from mammalian genes, and examples are enhancers from the genes that
35 encode mammalian globin, elastase, albumin, α -fetoprotein, and insulin. Enhancers for use in the compositions and methods described herein also include those that are derived from the genetic material of a virus capable of infecting a eukaryotic cell. Examples are the 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, and adenovirus enhancers. Additional enhancer

sequences that induce activation of eukaryotic gene transcription are disclosed in Yaniv et al., Nature 297:17 (1982). Another enhancer that may be used in the β LCR.

Cells for Expression and Delivery of C1-INH

5 Cells that may be used in conjunction with the compositions and methods described herein include cells that are capable of undergoing further differentiation. For example, one type of cell that can be used in conjunction with the compositions and methods described herein is a pluripotent cell. A pluripotent cell is a cell that possesses the ability to develop into more than one differentiated cell type. Examples of pluripotent cells are ESCs, iPSCs, and CD34+ cells. ESCs and iPSCs have the
10 ability to differentiate into cells of the ectoderm, which gives rise to the skin and nervous system, endoderm, which forms the gastrointestinal and respiratory tracts, endocrine glands, liver, and pancreas, and mesoderm, which forms bone, cartilage, muscles, connective tissue, and most of the circulatory system.

 Cells that may be used in conjunction with the compositions and methods described herein
15 include hematopoietic stem cells and hematopoietic progenitor cells. Hematopoietic stem cells (HSCs) are immature blood cells that have the capacity to self-renew and to differentiate into mature blood cells including diverse lineages including but not limited to granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes,
20 macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells). Human HSCs are CD34+. In addition, HSCs also refer to long term repopulating HSC (LT-HSC) and short-term repopulating HSC (ST-HSC). Any of these HSCs can be used in conjunction with the compositions and methods described herein.

 HSCs and other pluripotent progenitors can be obtained from blood products. A blood
25 product is a product obtained from the body or an organ of the body containing cells of hematopoietic origin. Such sources include unfractionated bone marrow, umbilical cord, placenta, peripheral blood, or mobilized peripheral blood. All of the aforementioned crude or unfractionated blood products can be enriched for cells having HSC or myeloid progenitor cell characteristics in a number of ways. For example, the more mature, differentiated cells can be selected against based on cell surface
30 molecules they express. The blood product may be fractionated by positively selecting for CD34+ cells, which include a subpopulation of hematopoietic stem cells capable of self-renewal, multipotency, and that can be re-introduced into a transplant recipient whereupon they home to the hematopoietic stem cell niche and reestablish productive and sustained hematopoiesis. Such selection is accomplished using, for example, commercially available magnetic anti-CD34 beads
35 (Dynal, Lake Success, NY). Myeloid progenitor cells can also be isolated based on the markers they express. Unfractionated blood products can be obtained directly from a donor or retrieved from cryopreservative storage. HSCs and myeloid progenitor cells can also be obtained from by differentiation of ES cells, iPS cells or other reprogrammed mature cell types.

Cells that may be used in conjunction with the compositions and methods described herein include allogeneic cells and autologous cells. When allogeneic cells are used, the cells may optionally be HLA-matched to the subject receiving a cell treatment.

Cells that may be used in conjunction with the compositions and methods described herein include CD34+/CD90+ cells and CD34+/CD164+ cells. These cells may contain a higher percentage of HSCs. These cells are described in Radtke et al. *Sci. Transl. Med.* 9: 1-10, 2017, and Pellin et al. *Nat. Comm.* 1-: 2395, 2019, the disclosures of each of which are hereby incorporated by reference in their entirety.

The cells described herein and above may be genetically modified so as to express C1-INH using, for example, a variety of methodologies (see, for example, the sections entitled "Methods of Producing Functional C1-INH-Expressing Cells by Viral Transduction," "Methods of Producing Functional C1-INH-Expressing Cells by Ex Vivo Transfection," and "Promoting Functional C1-INH Expression Using Gene Editing Techniques"). Once the cells have been adapted to express physiological levels of functional C1-INH, these cells have therapeutic utility, and are referred to herein as "therapeutic cells of the disclosure."

Promoting Functional C1-INH Expression Using Gene Editing Techniques

Another useful tool for the disruption and/or integration of target genes into the genome of a cell (e.g., a pluripotent stem cell) is the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system, a system that originally evolved as an adaptive defense mechanism in bacteria and archaea against viral infection. The CRISPR/Cas system includes palindromic repeat sequences within plasmid DNA and a CRISPR-associated protein (Cas; e.g., Cas9 or Cas12a). This ensemble of DNA and protein directs site specific DNA cleavage of a target sequence by first incorporating foreign DNA into CRISPR loci. Polynucleotides containing these foreign sequences and the repeat-spacer elements of the CRISPR locus are in turn transcribed in a host cell to create a guide RNA, which can subsequently anneal to a target sequence and localize the Cas nuclease to this site. In this manner, highly site-specific Cas-mediated DNA cleavage can be engendered in a foreign polynucleotide because the interaction that brings Cas within close proximity of the target DNA molecule is governed by RNA: DNA hybridization. As a result, one can design a CRISPR/Cas system to cleave any target DNA molecule of interest. This technique has been exploited in order to edit eukaryotic genomes (Hwang et al. *Nature Biotechnology* 31:227 (2013), the disclosure of which is incorporated herein by reference) and can be used as an efficient means of site-specifically editing pluripotent stem cell genomes in order to cleave DNA prior to the incorporation of a gene encoding a target gene. The use of CRISPR/Cas to modulate gene expression has been described in, e.g., WO 2017/182881 and US 8,697,359, the disclosures of each of which are incorporated herein by reference.

For example, using the compositions and methods of the disclosure, a genetic locus containing a nucleic acid that encodes a defective C1-INH protein may be edited so as to recapitulate functional C1-INH expression. A genetic locus in a target cell, such as an autologous cell obtained from a patient suffering from HAE, may be edited at a site near or within the gene encoding

endogenous C1-INH. The gene encoding endogenous C1-INH may be one, for example, that has a mutation causing a C1-INH defect. To edit the target cell genome at this site, the cell may be provided a nuclease, such as a CRISPR-associated protein described above, along with a guide RNA (gRNA) and a template nucleic acid that encodes functional C1-INH. The gRNA may direct the
5 nuclease to the desired site within the target cell genome that is within or near a gene encoding a defective C1-INH protein. This may be achieved, for example, by base pair hybridization between the gRNA and the desired site in the target cell genome. Upon hybridization between the gRNA and the desired site, the nuclease may then catalyze a single-strand break or double-strand break at the desired site. Following this cleavage event, the template nucleic acid encoding functional C1-INH
10 may then insert into the target cell genome at the desired site. In some embodiments, the template nucleic acid encoding functional C1-INH is inserted at a site that is operably joined to the endogenous C1-INH promoter, resulting in recapitulation of functional C1-INH protein expression.

Alternatively, base editing may be used to site-specifically edit one or more nucleobase at a desired site in the target cell genome so as to negate a C1-INH defect-causing mutation and
15 recapitulate expression of a gene encoding functional C1-INH. Base editing techniques may use, for example, a mutant Cas9 that induces a single-strand break in one strand of endogenous DNA in the target cell, at which point a fused deaminase then converts one base to another, such as adenine (A) to inosine (I), a proxy for guanine (G) following DNA replication. The accompanying T to C change in the remaining DNA strand occurs by way of DNA repair and replication. Base editing may also be
20 used at the level of RNA, as mutant Cas13-ADAR fusion proteins have been deployed to bind RNA and catalyzing nucleobase modifications resulting in a change of A to I. Exemplary methods for DNA base editing that may be used to negate a defect-causing C1-INH mutation in the cells and recapitulate expression of a functional C1-INH protein are described in Cohen, "Novel CRISPR-derived 'base editors' surgically alter DNA or RNA, offering new ways to fix mutations," Science
25 Magazine, October 2017, the disclosure of which is incorporated herein by reference.

Alternative methods for disruption of a target DNA by site-specifically cleaving genomic DNA prior to the incorporation of a gene of interest in a pluripotent stem cell include the use of zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Unlike the CRISPR/Cas system, these enzymes do not contain a guiding polynucleotide to localize to a specific
30 target sequence. Target specificity is instead controlled by DNA binding domains within these enzymes. The use of ZFNs and TALENs in genome editing applications is described, e.g., in Urnov et al. Nature Reviews Genetics 11:636 (2010); and in Joung et al. Nature Reviews Molecular Cell Biology 14:49 (2013), the disclosures of each of which are incorporated herein by reference. In some embodiments, an endogenous gene is disrupted, e.g., in a pluripotent stem cell, using the gene
35 editing techniques described above.

In some embodiments, a gene editing approach, such as a CRISPR/Cas system or another of the nucleases described above, is used in order to insert a gene encoding a functional C1-INH protein (i.e., a C1-INH protein lacking an activity-disrupting mutation) directly into an endogenous C1-INH locus in a cell obtained from a patient suffering from HAE. In this way, expression of mutant C1-INH
40 may be suppressed while simultaneously inducing expression of a functional C1-INH protein.

In some embodiments, a gene editing approach, such as a CRISPR/Cas system or another of the nucleases described above, is used in order to insert a gene encoding a functional C1-INH protein (i.e., a C1-INH protein lacking an activity-disrupting mutation) directly into a non-C1-INH locus in a cell obtained from a patient suffering from HAE. For example, the gene could be inserted in the AAVS1 locus or another safe harbor locus, e.g., as described in Papapetrou et al. *Mol Ther.*24:678-684, 2016, hereby incorporated by reference in its entirety.

Agents that Promote Pluripotent Cell Mobilization

In some embodiments of the disclosure, prior to isolation of a pluripotent cell from the subject being treated for HAE (e.g., in the case of an autologous cell population) or from a donor (e.g., in the case of an allogeneic cell population), the subject or donor is administered one or more mobilization agents that stimulate the migration of pluripotent cells (e.g., CD34+ HSCs and HPCs) from a stem cell niche, such as the bone marrow, to peripheral circulation. Exemplary cell mobilization agents that may be used in conjunction with the compositions and methods of the disclosure are described herein and known in the art. For example, the mobilization agent may be a C-X-C motif chemokine receptor (CXCR) 2 (CXCR2) agonist. The CXCR2 agonist may be Gro-beta, or a truncated variant thereof. Gro-beta and variants thereof are described, for example, in US Patent Nos. 6,080,398; 6,447,766; and 6,399,053, the disclosures of each of which are incorporated herein by reference in their entirety. Additionally, or alternatively, the mobilization agent may include a CXCR4 antagonist, such as plerixafor or a variant thereof. Plerixafor and structurally similar compounds are described, for example, in US Patent Nos. 6,987,102; 7,935,692; and 7,897,590, the disclosures of each of which are incorporated herein by reference. Additionally, or alternatively, the mobilization agent may include granulocyte colony-stimulating factor (G-CSF). The use of G-CSF as an agent to induce mobilization of pluripotent cells (e.g., CD34+ HSCs and/or HPCs) from a stem cell niche to peripheral circulation is described, for example, in US 2010/0178271, the disclosure of which is incorporated herein by reference in its entirety.

Agents that Enhance Cellular Engraftment

In some embodiments, the one or more agents administered to a patient that increase activity or expression of functional C1-INH is a population of cells (e.g., CD34+ cells) that express a C1-INH transgene. In such instances, prior to administration of the cells to the patient, the patient may be administered an agent that ablates an endogenous population of CD34+ cells, allowing the administered CD34+ cells to engraft in the patient. Examples of conditioning agents include myeloablative conditioning agents, which deplete a wide variety of hematopoietic cells in a patient. For instance, that patient may be pre-treated with an alkylating agent, such as a nitrogen mustard (e.g., bendamustine, chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, or melphalan), a nitrosourea (e.g., carmustine, lomustine, or streptozocin), an alkyl sulfonate (e.g., busulfan), a triazine (e.g., dacarbazine or temozolomide), or an ethylenimine (e.g., altretamine or thiotepa). In some embodiments, the patient is administered a conditioning agent that selectively ablates a specific population of endogenous cells, such as a population of endogenous CD34+ HSCs or HPCs.

In some embodiments, the conditioning agent includes an antibody or antigen-binding fragment thereof. The antibody or antigen-binding fragment thereof may bind to CD117, HLA-DR, CD34, CD90, CD45, or CD133 (e.g., CD117). The antibody or antigen-binding fragment thereof may be conjugated to a cytotoxin.

5 In some embodiments, the patient is pre-treated with an activator of prostaglandin E receptor signaling in order to help facilitate the engraftment of administered C1-INH-expressing cells. The prostaglandin E receptor signaling activator may be, for example, selected from the group consisting of: prostaglandin (PG) A2 (PGA2), PGB2, PGD2, PGE1 (Alprostadi), PGE2, PGF2, PGI2 (Epoprostenol), PGH2, PGJ2, and derivatives and analogs thereof.

10 In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is PGE2 or dmPG2.

In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is 15d-PGJ2, delta2-PGJ2, 2-hydroxyheptadecatrienoic acid (HHT), Thromboxane (TXA2 and TXB2), PGI2 analogs, e.g., Iloprost and Treprostinil, PGF2 analogs, e.g., Travoprost, Carboprost tromethamine, Tafluprost, Latanoprost, 15 Bimatoprost, Unoprostone isopropyl, Cloprostenol, Oestrophane, and Superphane, PGE1 analogs, e.g., 11-deoxy PGE1, Misoprostol, and Butaprost, and Corey alcohol-A ([3aa,4a,5,6aa]-(-)-[Hexahydro-4-(hydroxymethyl)-2-oxo-2H-cyclopenta[b]furan-5-yl][1,1'-biphenyl]-4-carboxylate), Corey alcohol-B (2H-Cyclopenta[b]furan-2-one,5-(benzoyloxy)hexahydro-4-(hydroxymethyl)[3aR-(3aa,4a,5,6aa)]), and 20 Corey diol ((3aR,4S,5R,6aS)-hexahydro-5-hydroxy-4-(hydroxymethyl)-2H-cyclopenta[b]furan-2-one).

In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is a prostaglandin E receptor ligand, such as prostaglandin E2 (PGE2), or an analog or derivative thereof. Prostaglandins refer generally to hormone-like molecules that are derived from fatty acids containing 20 carbon atoms, including a 5-carbon ring, as described herein and known in the art. Illustrative examples of PGE2 "analog" or "derivatives" include, but are not limited to, 16,16-dimethyl PGE2, 16-16 dimethyl PGE2 p-(p-acetamidobenzamido) phenyl ester, 11-deoxy-16,16-dimethyl PGE2, 9-deoxy-9-methylene-16, 16-dimethyl PGE2, 9-deoxy-9-methylene PGE2, 9-keto Fluprostenol, 5-trans PGE2, 17-phenyl- omega-trinor PGE2, PGE2 serinol amide, PGE2 methyl ester, 16-phenyl tetranor PGE2, 15(S)- 15-methyl PGE2, 15 (R)- 15-methyl PGE2, 8-iso-15-keto PGE2, 8-iso PGE2 isopropyl ester, 20-hydroxy PGE2, 30 nocloprost, sulprostone, butaprost, 15-keto PGE2, and 19 (R) hydroxy PGE2.

In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is a prostaglandin analog or derivative having a similar structure to PGE2 that is substituted with halogen at the 9-position (see, e.g., WO 2001/12596, 35 herein incorporated by reference in its entirety), as well as 2-decarboxy-2-phosphinico prostaglandin derivatives, such as those described in US 2006/0247214, herein incorporated by reference in its entirety).

In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is a non-PGE2-based ligand. In some 40 embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of

a C1-INH-expressing cell is CAY10399, ONO_8815Ly, ONO-AE1-259, or CP-533,536. Additional examples of non-PGE₂-based EP₂ agonists include the carbazoles and fluorenes disclosed in WO 2007/071456, herein incorporated by reference for its disclosure of such agents. Illustrative examples of non-PGE₂-based EP₃ agonist include, but are not limited to, AE5-599, MB28767, GR 63799X, ONO- NT012, and ONO-AE-248. Illustrative examples of non-PGE₂-based EP₄ agonist include, but are not limited to, ONO-4819, APS-999 Na, AH23848, and ONO-AE 1- 329. Additional examples of non-PGE₂-based EP₄ agonists can be found in WO 2000/038663; US Patent No. 6,747,037; and US Patent No. 6,610,719, each of which are incorporated by reference for their disclosure of such agonists

In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is a Wnt agonist. Illustrative examples of Wnt agonists include, but are not limited to, Wnt polypeptides and glycogen synthase kinase 3 (GSK3) inhibitors. Illustrative examples of Wnt polypeptides suitable for use as compounds that stimulate the prostaglandin EP receptor signaling pathway include, but are not limited to, Wnt1, Wnt2, Wnt2b/13, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt7c, Wnt8, Wnt8a, Wnt8b, Wnt8c, Wnt10a, Wnt10b, Wnt11, Wnt14, Wnt15, or biologically active fragments thereof. GSK3 inhibitors suitable for use as agents that stimulate the prostaglandin EP receptor signaling pathway bind to and decrease the activity of GSK3a, or GSK3. Illustrative examples of GSK3 inhibitors include, but are not limited to, BIO (6- bromindirubin-3'-oxime), LiCl, Li₂CO₃ or other GSK-3 inhibitors, as exemplified in US Patents Nos. 6,057,117 and 6,608,063, as well as US 2004/0092535 and US 2004/0209878, and ATP- competitive, selective GSK-3 inhibitors CHIR-911 and CHIR-837 (also referred to as CT-99021/CHIR-99021 and CT-98023/CHIR-98023, respectively) (Chiron Corporation (Emeryville, CA)).

In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is an agent that increases signaling through the cAMP/P13K/AKT second messenger pathway, such as an agent selected from the group consisting of dibutyl cAMP (DBcAMP), phorbol ester, forskolin, sclareline, 8-bromo-cAMP, cholera toxin (CTx), aminophylline, 2,4 dinitrophenol (DNP), norepinephrine, epinephrine, isoproterenol, isobutylmethylxanthine (IBMX), caffeine, theophylline (dimethylxanthine), dopamine, rolipram, iloprost, pituitary adenylate cyclase activating polypeptide (PACAP), and vasoactive intestinal polypeptide (VIP), and derivatives of these agents.

In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is an agent that increases signaling through the Ca²⁺ second messenger pathway, such as an agent selected from the group consisting of Bapta-AM, Fendiline, Nicardipine, and derivatives of these agents.

In some embodiments, the activator of prostaglandin E receptor signaling used to help facilitate engraftment of a C1-INH-expressing cell is an agent that increases signaling through the NO/ Angiotensin signaling, such as an agent selected from the group consisting of L-Arg, Sodium Nitroprusside, Sodium Vanadate, Bradykinin, and derivatives thereof.

Methods of Measuring C1-INH Gene Expression

Preferably, the compositions and methods of the disclosure are used to facilitate expression of functional C1-INH at physiologically normal levels in a patient (e.g., a human patient having HAE). The therapeutic agents of the disclosure, for example, may stimulate functional C1-INH expression in a human patient (e.g., a human patient suffering from HAE) that has a C1-INH deficiency. For example, the therapeutic agents of the disclosure may facilitate C1-INH expression in a HAE patient at a level of, for example, from about 20% to about 200% of the level of functional C1-INH expression observed in a human subject of comparable age and body mass index that does not have a C1-INH deficiency (e.g., about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195%, or 200% of the level of functional C1-INH expression observed in a human subject of comparable age and body mass index that does not have a C1-INH deficiency).

The expression level of functional C1-INH expressed in a patient can be ascertained, for example, by evaluating the concentration or relative abundance of mRNA transcripts derived from transcription of a functional C1-INH transgene. Additionally, or alternatively, gene expression can be determined by evaluating the concentration or relative abundance of functional C1-INH protein produced by transcription and translation of a C1-INH transgene. Protein concentrations can also be assessed using functional assays, such as MDP detection assays. The sections that follow describe exemplary techniques that can be used to measure the expression level of a C1-INH transgene upon delivery to a patient, such as a patient having HAE as described herein. Transgene expression can be evaluated by a number of methodologies known in the art, including, but not limited to, nucleic acid sequencing, microarray analysis, proteomics, in-situ hybridization (e.g., fluorescence in-situ hybridization (FISH)), amplification-based assays, in situ hybridization, fluorescence activated cell sorting (FACS), northern analysis and/or PCR analysis of mRNAs.

Nucleic acid detection

Nucleic acid-based methods for determining C1-INH transgene expression detection that may be used in conjunction with the compositions and methods described herein include imaging-based techniques (e.g., Northern blotting or Southern blotting). Such techniques may be performed using cells obtained from a patient following administration of the C1-INH transgene. Northern blot analysis is a conventional technique well known in the art and is described, for example, in Molecular Cloning, a Laboratory Manual, second edition, 1989, Sambrook, Fritsch, Maniatis, Cold Spring Harbor Press, 10 Skyline Drive, Plainview, NY 11803-2500. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel et al., eds., 1995, Current Protocols In Molecular Biology, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis).

Transgene detection techniques that may be used in conjunction with the compositions and methods described herein to evaluate C1-INH expression further include microarray sequencing experiments (e.g., Sanger sequencing and next-generation sequencing methods, also known as high-

throughput sequencing or deep sequencing). Exemplary next generation sequencing technologies include, without limitation, Illumina sequencing, Ion Torrent sequencing, 454 sequencing, SOLiD sequencing, and nanopore sequencing platforms. Additional methods of sequencing known in the art can also be used. For instance, transgene expression at the mRNA level may be determined using RNA-Seq (e.g., as described in Mortazavi et al., Nat. Methods 5:621-628 (2008) the disclosure of which is incorporated herein by reference in their entirety). RNA-Seq is a robust technology for monitoring expression by direct sequencing the RNA molecules in a sample. Briefly, this methodology may involve fragmentation of RNA to an average length of 200 nucleotides, conversion to cDNA by random priming, and synthesis of double-stranded cDNA (e.g., using the Just cDNA DoubleStranded cDNA Synthesis Kit from Agilent Technology). Then, the cDNA is converted into a molecular library for sequencing by addition of sequence adapters for each library (e.g., from Illumina®/Solexa), and the resulting 50-100 nucleotide reads are mapped onto the genome.

Transgene expression levels may be determined using microarray-based platforms (e.g., single-nucleotide polymorphism arrays), as microarray technology offers high resolution. Details of various microarray methods can be found in the literature. See, for example, U.S. Pat. No. 6,232,068 and Pollack et al., Nat. Genet. 23:41-46 (1999), the disclosures of each of which are incorporated herein by reference in their entirety. Using nucleic acid microarrays, mRNA samples are reverse transcribed and labeled to generate cDNA. The probes can then hybridize to one or more complementary nucleic acids arrayed and immobilized on a solid support. The array can be configured, for example, such that the sequence and position of each member of the array is known. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene. Expression level may be quantified according to the amount of signal detected from hybridized probe-sample complexes. A typical microarray experiment involves the following steps: 1) preparation of fluorescently labeled target from RNA isolated from the sample, 2) hybridization of the labeled target to the microarray, 3) washing, staining, and scanning of the array, 4) analysis of the scanned image and 5) generation of gene expression profiles. One example of a microarray processor is the Affymetrix GENECHIP® system, which is commercially available and comprises arrays fabricated by direct synthesis of oligonucleotides on a glass surface. Other systems may be used as known to one skilled in the art.

Amplification-based assays also can be used to measure the expression level of a transgene in a target cell following delivery to a patient. In such assays, the nucleic acid sequences of the gene act as a template in an amplification reaction (for example, PCR, such as qPCR). In a quantitative amplification, the amount of amplification product is proportional to the amount of template in the original sample. Comparison to appropriate controls provides a measure of the expression level of the gene, corresponding to the specific probe used, according to the principles described herein. Methods of real-time qPCR using TaqMan probes are well known in the art. Detailed protocols for real-time qPCR are provided, for example, in Gibson et al., Genome Res. 6:995-1001 (1996), and in Heid et al., Genome Res. 6:986-994 (1996), the disclosures of each of which are incorporated herein by reference in their entirety. Levels of gene expression as described herein can be determined by RT-PCR technology. Probes used for PCR may be labeled with a detectable marker, such as, for

example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator, or enzyme.

Protein detection

5 Transgene expression can additionally be determined by measuring the concentration or relative abundance of a corresponding protein product (e.g., C1-INH) encoded by a gene of interest. Protein levels can be assessed using standard detection techniques known in the art. Protein expression assays suitable for use with the compositions and methods described herein include proteomics approaches, immunohistochemical and/or western blot analysis, immunoprecipitation, molecular binding assays, ELISA, enzyme-linked immunofiltration assay (ELIFA), mass spectrometry, mass spectrometric immunoassay, and biochemical enzymatic activity assays. In particular, proteomics methods can be used to generate large-scale protein expression datasets in multiplex. Proteomics methods may utilize mass spectrometry to detect and quantify polypeptides (e.g., proteins) and/or peptide microarrays utilizing capture reagents (e.g., antibodies) specific to a panel of target proteins to identify and measure expression levels of proteins expressed in a sample (e.g., a single cell sample or a multi-cell population).

Exemplary peptide microarrays have a substrate-bound plurality of polypeptides, the binding of an oligonucleotide, a peptide, or a protein to each of the plurality of bound polypeptides being separately detectable. Alternatively, the peptide microarray may include a plurality of binders, including, but not limited to, monoclonal antibodies, polyclonal antibodies, phage display binders, yeast two-hybrid binders, aptamers, which can specifically detect the binding of specific oligonucleotides, peptides, or proteins. Examples of peptide arrays may be found in U.S. Patent Nos. 6,268,210, 5,766,960, and 5,143,854, the disclosures of each of which are incorporated herein by reference in their entirety.

25 Mass spectrometry (MS) may be used in conjunction with the methods described herein to identify and characterize transgene expression in a cell from a patient (e.g., a human patient) following delivery of the transgene. Any method of MS known in the art may be used to determine, detect, and/or measure a protein or peptide fragment of interest, e.g., LC-MS, ESI-MS, ESI-MS/MS, MALDI-TOF-MS, MALDI-TOF/TOF-MS, tandem MS, and the like. Mass spectrometers generally contain an ion source and optics, mass analyzer, and data processing electronics. Mass analyzers include scanning and ion-beam mass spectrometers, such as time-of-flight (TOF) and quadrupole (Q), and trapping mass spectrometers, such as ion trap (IT), Orbitrap, and Fourier transform ion cyclotron resonance (FT-ICR), may be used in the methods described herein. Details of various MS methods can be found in the literature. See, for example, Yates et al., Annu. Rev. Biomed. Eng. 11:49-79, 2009, the disclosure of which is incorporated herein by reference in its entirety.

Prior to MS analysis, proteins in a sample obtained from the patient can be first digested into smaller peptides by chemical (e.g., via cyanogen bromide cleavage) or enzymatic (e.g., trypsin) digestion. Complex peptide samples also benefit from the use of front-end separation techniques, e.g., 2D-PAGE, HPLC, RPLC, and affinity chromatography. The digested, and optionally separated, sample is then ionized using an ion source to create charged molecules for further analysis.

Ionization of the sample may be performed, e.g., by electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), photoionization, electron ionization, fast atom bombardment (FAB)/liquid secondary ionization (LSIMS), matrix assisted laser desorption/ionization (MALDI), field ionization, field desorption, thermospray/plasmaspray ionization, and particle beam ionization.

5 Additional information relating to the choice of ionization method is known to those of skill in the art.

After ionization, digested peptides may then be fragmented to generate signature MS/MS spectra. Tandem MS, also known as MS/MS, may be particularly useful for analyzing complex mixtures. Tandem MS involves multiple steps of MS selection, with some form of ion fragmentation occurring in between the stages, which may be accomplished with individual mass spectrometer
10 elements separated in space or using a single mass spectrometer with the MS steps separated in time. In spatially separated tandem MS, the elements are physically separated and distinct, with a physical connection between the elements to maintain high vacuum. In temporally separated tandem MS, separation is accomplished with ions trapped in the same place, with multiple separation steps taking place over time. Signature MS/MS spectra may then be compared against a peptide sequence
15 database (e.g., SEQUEST). Post-translational modifications to peptides may also be determined, for example, by searching spectra against a database while allowing for specific peptide modifications.

Routes of Administration

The compositions described herein may be administered to a patient (e.g., a human patient
20 suffering from HAE) by one or more of a variety of routes, such as intravenously or by means of a bone marrow transplant. The most suitable route for administration in any given case may depend on the particular composition administered, the patient, pharmaceutical formulation methods, administration methods (e.g., administration time and administration route), the patient's age, body weight, sex, severity of the diseases being treated, the patient's diet, and the patient's excretion rate.
25 Multiple routes of administration may be used to treat a single patient at one time, or the patient may receive treatment via one route of administration first and receive treatment via another route of administration during a second appointment, e.g., 1 week later, 2 weeks later, 1 month later, 6 months later, or 1 year later. Compositions may be administered to a subject once, or cells may be administered one or more times (e.g., 2-10 times) per week, month, or year.

30

Selection of Donor Cells

In some embodiments, the patient undergoing treatment is the donor that provides cells (e.g., pluripotent cells, such as CD34+ hematopoietic stem or progenitor cells) that are subsequently modified to express one or more therapeutic proteins of the disclosure before being re-administered
35 to the patient. In such cases, withdrawn cells (e.g., hematopoietic stem or progenitor cells) may be re-infused into the subject following, for example, incorporation of a transgene encoding functional C1-INH, such that the cells may subsequently home to hematopoietic tissue and establish productive hematopoiesis, thereby populating or repopulating a line of cells that is defective or deficient in the patient. In cases in which the patient undergoing treatment also serves as the cell donor, the
40 transplanted cells (e.g., hematopoietic stem or progenitor cells) are less likely to undergo graft

rejection. This stems from the fact that the infused cells are derived from the patient and express the same HLA class I and class II antigens as expressed by the patient. Alternatively, the patient and the donor may be distinct. In some embodiments, the patient and the donor are related, and may, for example, be HLA-matched. As described herein, HLA-matched donor-recipient pairs have a
5 decreased risk of graft rejection, as endogenous T cells and NK cells within the transplant recipient are less likely to recognize the incoming hematopoietic stem or progenitor cell graft as foreign and are thus less likely to mount an immune response against the transplant. Exemplary HLA-matched donor-recipient pairs are donors and recipients that are genetically related, such as familial donor-recipient pairs (e.g., sibling donor-recipient pairs). In some embodiments, the patient and the donor
10 are HLA-mismatched, which occurs when at least one HLA antigen, in particular with respect to HLA-A, HLA-B and HLA-DR, is mismatched between the donor and recipient. To reduce the likelihood of graft rejection, for example, one haplotype may be matched between the donor and recipient, and the other may be mismatched.

15 **Pharmaceutical Compositions and Dosing**

In cases in which a patient is administered a population of cells that together express one or more therapeutic proteins of the disclosure, the number of cells administered may depend, for example, on the expression level of the desired protein(s), the patient, pharmaceutical formulation methods, administration methods (e.g., administration time and administration route), the patient's
20 age, body weight, sex, severity of the disease being treated, and whether or not the patient has been treated with agents to ablate endogenous pluripotent cells (e.g., endogenous CD34+ cells, hematopoietic stem or progenitor cells, or microglia, among others). The number of cells administered may be, for example, from 1×10^6 cells/kg to 1×10^{12} cells/kg, or more (e.g., 1×10^7 cells/kg, 1×10^8 cells/kg, 1×10^9 cells/kg, 1×10^{10} cells/kg, 1×10^{11} cells/kg, 1×10^{12} cells/kg, or more).
25 Cells may be administered in an undifferentiated state, or after partial or complete differentiation into microglia. The number of pluripotent cells may be administered in any suitable dosage form.

Cells may be admixed with one or more pharmaceutically acceptable carriers, diluents, and/or excipients. Exemplary carriers, diluents, and excipients that may be used in conjunction with the compositions and methods of the disclosure are described, e.g., in Remington: The Science and
30 Practice of Pharmacy (2012, 22nd ed.) and in The United States Pharmacopeia: The National Formulary (2015, USP 38 NF 33).

Examples

The following examples are put forth so as to provide those of ordinary skill in the art with a
35 description of how the compositions and methods described herein may be used, made, and evaluated, and are intended to be purely exemplary of the disclosure and are not intended to limit the scope of what the inventors regard as their disclosure.

Example 1. SERPING1 is expressed in HT29 and K562 cell lines following transduction*Materials and Methods*

Wild-type or codon optimized C1-INH genes were synthesized by ThermoFisher using the proprietary GeneArt technology platform. Promoters, nucleic acid, and posttranscriptional regulatory
 5 minimal elements were ligated into a plasmid of interest using standard molecular cloning techniques with suitable restriction endonuclease-mediated cleavage and ligation protocols. Additional elements may be included using PCR-based techniques, Gibson assembly and further cloning procedures known in the art.

Transfer lentiviral vector plasmids were used to produce lentiviral particles in adherent
 10 HEK293T cell line with TransIT-VirusGEN Transfection reagent (Mirus) combining Gag-Pol, Rev, VSV-G and transfer vector following manufacturer protocol. After harvesting and concentration of produced lentiviral particles, virus titration was performed in HT29 cell line. Transducing units per milliliter were calculated by means of droplet digital PCR containing HT29 genomic DNA as template, ddPCR supermix for probes (Bio-Rad), Hiv Psi as target and RNaseP as reference gene.

15 HT29 and K562 were transduced with wild-type or codon optimized C1-INH lentiviral vector constructs generated for ex vivo HSC transduction at different Multiplicity of Infections (MOIs) and cells collected at different time points.

RNA was extracted from transduced and untransduced control cells with RNeasy micro kit (Qiagen). iScript Reverse transcription Supermix for RT-qPCR from Bio-Rad was used for RNA
 20 reverse transcription in first strand cDNA following the manufacturer reaction protocol. Multiplex RT-PCR was performed using ssoAdvanced universal probes 2x supermix (Bio-Rad), wt SERPING1 FAM as target and RNaseP VIC as endogenous control and run on CFX384 Touch Real-Time PCR Detection System machine. Data plotted in FIGS. 3A-3B were analysed with Bio-Rad CFX Manager software.

25 Western blot to detect C1 inhibitor protein levels was evaluated in whole cell lysates from HT29 cells transduced with LV vectors. HT29 cells were harvested 4 days after transduction and lysed in RIPA buffer containing protease inhibitors. Whole cellular lysates (10µg for each sample) were separated on a 4-20% precast polyacrylamide gel (Bio-Rad) and transferred onto a nitrocellulose membrane using Trans-Blot Turbo Transfer system (Bio-Rad). Membranes were pre-
 30 blocked 1 hour with 5% Milk TBS-tween and then incubated with the following primary antibodies: mouse monoclonal anti-SERPING1 antibody 1:500 1 hour at room temperature or with rabbit polyclonal anti-β-actin primary antibody 1: 2000 for 1 hour at room temperature. Anti-mouse and anti-rabbit HRP-conjugated secondary antibodies were diluted 1:2000 and incubated 1h at RT. Immobilon Western Chemiluminescent HRP Substrate (Millipore) was used to detect signal and images are
 35 acquired with ChemiDoc Imaging System (Bio-Rad).

Results

FIG. 1 shows a therapeutic lentiviral vector construct generated for ex vivo HSC transduction. WT or codon optimized versions of human SERPING1 gene expression is under constitutive

Elongation Factor 1 alpha core promoter. SERPING1 coding sequences are combined with a WPRE posttranscriptional regulatory element to enhance expression.

FIGS. 2A and 2B show therapeutic lentiviral vectors generated for *in vivo* transduction construct features. Shown are constructs with wild type (FIG. 2A) or codon optimized version (FIG. 2B) of human SERPING1 under enhanced transthyretin promoter control. Wild type or codon optimized SERPING1 coding sequences are combined with WPRE Posttranscriptional Regulatory Element to enhance expression. Additional vectors combined with 2A cleavage peptide and luciferase were generated to evaluate biodistribution.

FIGS. 3A and 3B are graphs showing real time RT-PCR evaluating WT SERPING1 gene expression in HT29 cells (FIG. 3A) and K562 cells (FIG. 3B). HT29 and K562 cells were transduced with wtSERPING1 lentiviral vector at different multiplicity of infection (MOIs 1-100). 150 ng of RNA extracted at day 4 post-transduction was used for reverse transcription to produce complementary DNA. Multiplex RT-PCR was performed using RNaseP as endogenous control. FIGS. 3A and 3B summarize relative expression of wtSERPING1 mRNA in HT29 and K562 cells, respectively. Relative expression of wtSERPING1 was normalized to an untransduced sample (UT). Statistical analysis was performed with a T-test for unpaired samples and values reported as mean of three (HT29) or two (K562) technical replicates $\pm$ SEM; ****P $\leq$ 0.0001 vs UT ** P $\leq$ 0.01 vs UT.

FIG. 4 shows a western blot analysis of SERPING1 protein levels in whole cell lysates from HT29 cells transduced with LV vectors. HT29 cells were harvested 4 days after transduction and lysed in RIPA buffer. Whole cellular lysates (10 μ g for each sample) were separated on a 4-20% precast polyacrylamide gel and transferred onto a nitrocellulose membrane. Membranes were pre-blocked and then incubated with the following primary antibodies: mouse monoclonal anti-SERPING1 antibody or with rabbit polyclonal anti- β -actin primary antibody as loading control. The expected band of 44KDa for SERPING1 was observed together with additional bands corresponding to predicted glycosylated forms of SERPING1. Glycosylated bands intensity was stronger in samples transduced with codon optimized version of SERPING1 and increased with higher Multiplicity of Infections (MOIs). UT, untransduced HT29 cells.

Example 2. Confirmation of SERPING1 expression following LV transduction

FIG. 5 is a graph showing the results from an ELISA assay used to evaluate functional C1 inhibitor levels in serum-free supernatant from a K562 cell line transduced with LV-SERPING1. K562 cells were seeded at a density of 1 million cells/ml overnight in serum-free conditions and conditioned supernatant from the same number of cells was collected the subsequent day. Different dilutions of supernatant were tested with MicroVue C1-Inhibitor Plus EIA for human plasma and serum. The assay was used to detect functional C1 inhibitor. Tests were performed following manufacturer protocol with minor modifications for cell culture supernatant. The level of C1 inhibitor in the sample was measured against the standards provided and compared with normal and abnormal plasma controls. Functional C1 inhibitor was undetectable in untransduced cells but was present in different dilutions of LV-SERPING1 transduced samples. This indicates that the protein was properly secreted.

FIG. 6 is a graph showing the evaluation of endogenous levels of SERPING1 in primary cells and cell lines, including HSC and sorted peripheral blood subsets. Preliminary tests to evaluate endogenous level of SERPING1 for subsequent assays were performed by means of Real Time RT-PCR. 150 ng of RNA were reverse transcribed to produce complementary DNA. Multiplex Real Time RT-PCR was performed using RNaseP as endogenous control. The plot shows delta cycle threshold (Ct) calculated as the difference between SERPING1 and RNaseP endogenous control respective Cts. Lower numbers indicate a higher expression of SERPING1.

FIG. 7A is a graph showing high efficiency LV transduction of CD34+ cells with codon optimized (CO) and WT SERPING1 LV vectors. The graph shows an average vector copy number in LV SERPING1 transduced cells with wild type and codon optimized SERPING1 (MOI 50 in the presence of transduction enhancers) from 3 different experiments. Cells were collected at day 12 of liquid culture and 50 ng of extracted gDNA was used to perform multiplex ddPCR (detection of Fold increase in mRNA levels relative to untransduced wtSERPING1 expression) with HivPsi to evaluate LV integration together with RNaseP as endogenous control.

FIG. 7B is a graph showing transduction of CD34+ cells with wild type and codon optimized SERPING1 LV increased expression by ~13 and ~45fold respectively, relative to endogenous SERPING1 expression. The graph shows mRNA absolute quantification with ddPCR for wild type and codon optimized SERPING1 in LV SERPING1. Briefly, 150 ng of RNA were reverse transcribed to produce complementary DNA. Multiplex ddPCR was performed using RNaseP as endogenous control testing different dilution of cDNA. wtSERPING1 and coSERPING1 copies/ μ l (mRNA) were normalized against endogenous mRNA wtSERPING1 level in untransduced cells. The plot depicts a 45-fold increase in expression in LV coSERPING1 sample.

FIG. 8 is a picture of a gel showing results from a western blot analysis of SERPING1 protein levels in whole cell lysates from CD34+ cells transduced with LV codon optimized SERPING1.

CD34+ cells were harvested 13 days after transduction and lysed in RIPA buffer. Whole cellular lysates (15 μ g for each sample) were separated on a 4-20% precast polyacrylamide gel and transferred onto nitrocellulose membrane. Membranes were pre-blocked and then incubated with the following primary antibodies: mouse monoclonal anti-SERPING1 antibody or with rabbit polyclonal anti- β -actin primary antibody as loading control. SERPING1 bands were observed only in LV SERPING1 sample with an additional band corresponding to predicted glycosylated form (105 KDa) of SERPING1.

FIGS. 9A-9C are a set of graphs showing that SERPING-1 HSC gene therapy results significant increase in levels of functional serum C1-Inhibitor production in a xenotransplant model. 7-week-old NSG-SGM3 mice were irradiation conditioned (2Gy gamma-irradiation) and transplanted with 0.75×10^6 gene modified CD34+ cells 4 hours later by intravenous injection. Transplanted cells were generated by transduction (MOI 50, with transduction enhancers) of CD34+ cells isolated from mobilized peripheral blood (healthy donor) with LV-SERPING1 or LV-SERPING1-Luciferase (Luci). FIG. 9A shows *in vitro* characterization of vector copy number (VCN) and transduction efficiency of donor CD34+ cells, with LV-SERPING1. FIG. 9B shows analysis of human chimerism in bone marrow of xenografted NSG-SGM3 mice, and differentiation of CD11b+CD14+ myeloid subsets *in*

vitro, in the presence and absence of ectopic expression of SERPING-1 by donor CD34+ cells. FIG. 9C shows serum levels of human functional C1-Inhibitor production achieved by LV-SERPING-1 and LV-SERPING1-Luci gene modified CD34+ cells, compared to endogenous expression by untransduced CD34+ cells (UNTRD).

5

Example 3. Generation of a pluripotent stem cell expressing functional C1-INH for the treatment of HAE

An exemplary method for making pluripotent cells (e.g., embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or CD34+ cells) that express functional C1-INH is by way of transduction. Retroviral vectors (e.g., a lentiviral vector, alpharetroviral vector, or gammaretroviral vector) containing, e.g., a suitable promoter, such as a promoter described herein, and a polynucleotide encoding functional C1-INH can be engineered using vector production techniques described herein or known in the art. After the retroviral vector is engineered, the retrovirus can be used to transduce pluripotent cells (e.g., ESCs, iPSCs, or CD34+ cells) to generate a population of pluripotent cells that express functional C1-INH.

Additional exemplary methods for making pluripotent cells that express functional C1-INH are transfection techniques. Using molecular biology procedures described herein and known in the art, plasmid DNA containing a promoter and a polynucleotide encoding functional C1-INH can be produced. For example, a nucleic acid encoding functional C1-INH may be amplified from a human cell line using PCR-based techniques known in the art, or a nucleic acid encoding functional C1-INH may be synthesized, for example, using solid-phase polynucleotide synthesis procedures. The nucleic acid and promoter can then be ligated into a plasmid of interest, for example, using suitable restriction endonuclease-mediated cleavage and ligation protocols. After the plasmid DNA is engineered, the plasmid can be used to transfect the pluripotent cells (e.g., ESCs, iPSCs, or CD34+ cells) using, for example, electroporation or another transfection technique described herein to generate a population of pluripotent cells that express the encoded protein(s).

Example 4. Administration of a therapeutic composition to a patient suffering from HAE

According to the methods disclosed herein, a patient, such as a human patient, can be treated so as to reduce or alleviate symptoms of HAE and/or so as to target an underlying biochemical etiology of the disease. To this end, the patient may be administered, for example, a population of pluripotent cells, (e.g., ESCs, iPSCs, CD34+ cells) expressing functional C1-INH. The population of pluripotent cells may be administered to the patient, for example, systemically (e.g., intravenously). The cells may be administered in a therapeutically effective amount, such as from 1×10^6 cells/kg to 1×10^{12} cells/kg or more (e.g., 1×10^7 cells/kg, 1×10^8 cells/kg, 1×10^9 cells/kg, 1×10^{10} cells/kg, 1×10^{11} cells/kg, 1×10^{12} cells/kg, or more).

Before the population of cells is administered to the patient, one or more agents may be administered to the patient to ablate the patient's endogenous hematopoietic cell population, for example, by administration of a conditioning agent described herein.

The success of the treatment may be monitored by way of various clinical indicators. Effective treatment of HAE using a composition of the disclosure may manifest, for example, as sustained disease remission, such as sustained disease remission for at least one year; an observation that the patient does not exhibit an angioedema attack for a period of from about two months to about one year; a serum concentration of at least about 7 mg/dl (e.g., from about 15 mg/dl to about 35 mg/dl; a serum concentration of C1-INH that is from about 40% to about 60% of a serum concentration of C1-INH protein exhibited by a subject that does not have HAE (e.g., a subject that is the same gender as the patient and/or (has the same body mass index as the patient); or a reduction of risk of suffocation due to laryngeal angioedema attacks.

10

Other Embodiments

Various modifications and variations of the described disclosure will be apparent to those skilled in the art without departing from the scope and spirit of the disclosure. Although the disclosure has been described in connection with specific embodiments, it should be understood that the disclosure as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the disclosure that are obvious to those skilled in the art are intended to be within the scope of the disclosure.

15

Other embodiments are in the claims.

CLAIMS

1. A method of treating hereditary angioedema (HAE) in a patient in need thereof, the method comprising administering to the patient a population of pluripotent cells comprising a transgene that encodes a C1-esterase inhibitor (C1-INH) protein.

2. A method of inducing sustained remission of HAE in a patient in need thereof, the method comprising administering to the patient a population of pluripotent cells comprising a transgene that encodes a C1-INH protein.

3. A method of preventing angioedema attacks in a patient diagnosed as having HAE, the method comprising administering to the patient a population of pluripotent cells comprising a transgene that encodes a C1-INH protein.

4. A method of reducing the risk of recurrent angioedema attacks in a patient diagnosed as having HAE, the method comprising administering to the patient a population of pluripotent cells comprising a transgene that encodes a C1-INH protein.

5. The method of claim 3 or 4, wherein the angioedema attacks occur in the patient's skin, mucosa, gastrointestinal tract, and/or genitourinary region.

6. A method of reducing the risk of developing laryngeal angioedema attacks in a patient diagnosed as having HAE, the method comprising administering to the patient a population of pluripotent cells comprising a transgene that encodes a C1-INH protein.

7. The method of any one of claims 1-6, wherein the pluripotent cells are hematopoietic stem cells (HSCs) or hematopoietic progenitor cells (HPCs).

8. The method of any one of claims 1-6, wherein the pluripotent cells are embryonic stem cells.

9. The method of any one of claims 1-6, wherein the pluripotent cells are induced pluripotent stem cells.

10. The method of any one of claims 1-7, wherein the pluripotent cells are CD34+ cells.

11. The method of claim 10, wherein the CD34+ cells are myeloid progenitor cells.

12. The method of any one of claims 1-11, wherein the population of pluripotent cells is administered systemically to the patient.

13. The method of claim 12, wherein the population of pluripotent cells is administered to the patient by way of intravenous injection.

14. The method of any one of claims 1-13, wherein the pluripotent cells are autologous with respect to the patient.

15. The method of any one of claims 1-13, wherein the pluripotent cells are allogeneic with respect to the patient.

16. The method of claim 15, wherein the pluripotent cells are HLA-matched to the patient.

17. The method of any one of claims 1-16, wherein the cells are transduced ex vivo to express C1-INH.

18. The method of claim 17, wherein the cells are transduced with a viral vector selected from the group consisting of a Retroviridae family virus, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, and a poxvirus.

19. The method of claim 18, wherein the viral vector is a Retroviridae family viral vector.

20. The method of claim 19, wherein the Retroviridae family viral vector is a lentiviral vector.

21. The method of claim 19, wherein the Retroviridae family viral vector is an alpharetroviral vector or a gammaretroviral vector.

22. The method of any one of claims 18-21, wherein the Retroviridae family viral vector comprises a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

23. The method of any one of claims 18-22, wherein the viral vector is a pseudotyped viral vector.

24. The method of claim 23, wherein the pseudotyped viral vector selected from the group consisting of a pseudotyped adenovirus, a pseudotyped parvovirus, a pseudotyped coronavirus, a pseudotyped rhabdovirus, a pseudotyped paramyxovirus, a pseudotyped picornavirus, a pseudotyped alphavirus, a pseudotyped herpes virus, a pseudotyped poxvirus, and a pseudotyped Retroviridae family virus.

25. The method of claim 24, wherein the pseudotyped viral vector is a lentiviral vector.

26. The method of any one of claims 23-25, wherein the pseudotyped viral vector comprises one or more envelope proteins from a virus selected from vesicular stomatitis virus (VSV), RD114 virus, murine leukemia virus (MLV), feline leukemia virus (FeLV), Venezuelan equine encephalitis virus (VEE), human foamy virus (HFV), walleye dermal sarcoma virus (WDSV), Semliki Forest virus (SFV), Rabies virus, avian leukosis virus (ALV), bovine immunodeficiency virus (BIV), bovine leukemia virus (BLV), Epstein-Barr virus (EBV), Caprine arthritis encephalitis virus (CAEV), Sin Nombre virus (SNV), Cherry Twisted Leaf virus (ChTLV), Simian T-cell leukemia virus (STLV), Mason-Pfizer monkey virus (MPMV), squirrel monkey retrovirus (SMRV), Rous-associated virus (RAV), Fujinami sarcoma virus (FuSV), avian carcinoma virus (MH2), avian encephalomyelitis virus (AEV), Alfa mosaic virus (AMV), avian sarcoma virus CT10, and equine infectious anemia virus (EIAV).

27. The method of claim 26, wherein the pseudotyped viral vector comprises a VSV-G envelope protein.

28. The method of any one of claims 1-16, wherein the pluripotent cells are transfected ex vivo to express C1-INH.

29. The method of claim 28, wherein the pluripotent cells are transfected using a cationic polymer, diethylaminoethyl dextran, polyethylenimine, a cationic lipid, a liposome, calcium phosphate, an activated dendrimer, and/or a magnetic bead.

30. The method of claim 28 or 29, wherein the pluripotent cells are transfected by way of electroporation, Nucleofection, squeeze-poration, sonoporation, optical transfection, Magnetofection, and/or impalefection.

31. The method of any one of claims 1-16, wherein the pluripotent cells are obtained by delivering to the cells a nuclease that catalyzes a single-strand break or a double-strand break at a target position within the genome of the cell, optionally wherein the target position is near or within a gene encoding an endogenous C1-INH protein.

32. The method of claim 31, wherein the nuclease is delivered to the cells in combination with a guide RNA (gRNA) that hybridizes to the target position within the genome of the cell.

33. The method of claim 31 or 32, wherein the nuclease is a clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein.

34. The method of claim 33, wherein the CRISPR-associated protein is CRISPR-associated protein 9 (Cas9) or CRISPR-associated protein 12a (Cas12a).

35. The method of claim 31 or 32, wherein the nuclease is a transcription activator-like effector nuclease, a meganuclease, or a zinc finger nuclease.

36. The method of any one of claims 31-35, wherein the cells are additionally contacted with a template nucleic acid encoding C1-INH while the cells are contacted with the nuclease.

37. The method of claim 36, wherein the template nucleic acid molecule encoding C1-INH comprises a 5' homology arm and a 3' homology arm having nucleic acid sequences that are sufficiently similar to the nucleic acid sequences located 5' to the target position and 3' to the target position, respectively, to promote homologous recombination.

38. The method of claim 36 or 37, wherein the nuclease, gRNA, and/or template nucleic acid are delivered to the cells by contacting the cells with a viral vector that encodes the nuclease, gRNA, and/or template nucleic acid.

39. The method of claim 38, wherein the viral vector that encodes the nuclease, gRNA, and/or template nucleic acid is an AAV, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, a poxvirus, or a Retroviridae family virus.

40. The method of claim 39, wherein the viral vector that encodes the nuclease, gRNA, and/or template nucleic acid is a Retroviridae family virus.

41. The method of claim 40, wherein the Retroviridae family virus is a lentiviral vector, alpharetroviral vector, or gammaretroviral vector.

42. The method of claim 40 or 41, wherein the Retroviridae family virus that encodes the nuclease, gRNA, and/or template nucleic acid comprises a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

43. The method of claim 38, wherein the viral vector that encodes the nuclease, gRNA, and/or template nucleic acid is an integration-deficient lentiviral vector.

44. The method of claim 38, wherein the viral vector that encodes the nuclease, gRNA, and/or template nucleic acid is an AAV selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, and AAVrh74.

45. The method of any one of claims 1-44, wherein prior to administering the population of pluripotent cells to the patient, a population of precursor cells is isolated from the patient or a donor,

and wherein the precursor cells are expanded ex vivo to yield the population of cells being administered to the patient.

46. The method of claim 45, wherein the precursor cells are CD34+ HSCs, and wherein the precursor cells are expanded without substantial loss of HSC functional potential.

47. The method of claim 45 or 46, wherein prior to isolation of the precursor cells from the patient or donor, the patient or donor is administered one or more pluripotent cell mobilization agents.

48. The method of any one of claims 1-47, wherein prior to administering the population of pluripotent cells to the patient, a population of endogenous pluripotent cells is ablated in the patient by administration of one or more conditioning agents to the patient.

49. The method of any one of claims 1-47, the method comprising ablating a population of endogenous pluripotent cells in the patient by administering to the patient one or more conditioning agents prior to administering the population of pluripotent cells to the patient.

50. The method of claim 48 or 49, wherein the one or more conditioning agents are non-myeloablative conditioning agents.

51. The method of any one of claims 48-50, wherein the one or more conditioning agents deplete a population of CD34+ cells in the patient.

52. The method of claim 51, wherein the depleted CD34+ cells are myeloid progenitor cells.

53. The method of any one of claims 48-52, wherein the one or more conditioning agents comprise an antibody or antigen-binding fragment thereof.

54. The method of claim 53, wherein the antibody or antigen-binding fragment thereof binds to CD117, HLA-DR, CD34, CD90, CD45, or CD133.

55. The method of claim 54, wherein the antibody or antigen-binding fragment thereof binds to CD117.

56. The method of any one of claims 53-55, wherein the antibody or antigen-binding fragment thereof is conjugated to a cytotoxin.

57. The method of any one of claims 1-56, wherein upon administration of the population of pluripotent cells to the patient, the administered cells, or progeny thereof, differentiate into one or more cell types selected from megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells,

myeoblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T-lymphocytes, and B-lymphocytes.

58. The method of any one of claims 1-57, wherein the transgene is operably linked to a ubiquitous promoter.

59. The method of any one of claims 1-57, wherein the transgene is operably linked to a tissue-specific promoter.

60. The method of any one of claims 1-57, wherein the transgene is operably linked to a myeloid cell-specific promoter.

61. The method of any one of claims 1-57, wherein the transgene is operably linked to a CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, elongation factor 1 α (EF1 α) promoter, EF1 α short form (EFS) promoter, phosphoglycerate kinase (PGK) promoter, α -globin promoter, β -globin promoter, DC172 promoter, human serum albumin promoter, alpha1 antitrypsin promoter, thyroxine binding globulin promoter, or C1-INH promoter.

62. The method of any one of claims 1-61, wherein the transgene is operably linked to an enhancer.

63. The method of claim 62, wherein the enhancer comprises a β -globin locus control region (β LCR).

64. The method of any one of claims 1-63, wherein the transgene is operably linked to a miRNA targeting sequence.

65. The method of claim 64, wherein the miRNA targeting sequence has complementarity to a miRNA that is endogenously expressed in a tissue in which expression of C1-INH is undesirable.

66. The method of any one of claims 1-65, wherein the patient is a mammal and the cells are mammalian cells.

67. The method of claim 66, wherein the mammal is a human and the cells are human cells.

68. The method of any one of claims 1-67, wherein the patient has a loss-of-function mutation in an endogenous gene encoding C1-INH.

69. The method of claim 68, wherein the mutation is a deletion or substitution of an amino acid located within the reactive center loop (RCL) of C1-INH.

70. The method of claim 68, wherein the mutation is a deletion or substitution of K251.

71. The method of claim 68, wherein the mutation is selected from the group consisting of A436T, R444H, R444C, R444S, V432E, A443V, Y199TER, I462S, and R378C.

72. The method of any one of claims 1-71, wherein the patient has a mutation in an endogenous gene encoding C1-INH that causes (i) a deletion or (ii) expression of a truncated transcript.

73. The method of any one of claims 1-72, wherein the patient has a mutation in a gene encoding coagulation factor XII (F12).

74. The method of any one of claims 68-73, wherein the mutation is heterozygous.

75. The method of any one of claims 68-73, wherein the mutation is homozygous.

76. The method of any one of claims 1-75, wherein the patient has previously been treated with one or more immunosuppressive agents, biologic agents, and/or corticosteroids.

77. The method of claim 76, wherein the patient has not responded to treatment with the one or more immunosuppressive agents, biologic agents, and/or corticosteroids.

78. The method of any one of claims 1-77, wherein the patient has previously been treated with one or more therapeutic agents selected from the group consisting of C1-esterase inhibitor, icatibant, and ecallantide.

79. The method of claim 78, wherein the patient has not responded to treatment with the one or more therapeutic agents.

80. The method of any one of claims 1-79, wherein the patient has previously been treated with one or more prophylactic agents selected from the group consisting of Cinryze, Haegarda, Takhzyro, and an androgen.

81. The method of claim 80, wherein the patient has not responded to treatment with the one or more prophylactic agents.

82. The method of any one of claims 1-81, wherein the patient is less than 12 years old.

83. The method of claim 82, wherein the patient is less than 6 years old.
84. The method of any one of claims 1-81, wherein the patient is more than 6 years old.
85. The method of claim 84, wherein the patient is more than 12 years old.
86. The method of any one of claims 1-85, wherein prior to administering the population of pluripotent cells to the patient, the patient exhibits angioedema attacks with a frequency of from one to ten times per month.
87. The method of any one of claims 1-85, wherein prior to administering the population of pluripotent cells to the patient, the patient exhibits angioedema attacks with a frequency of one or two times per week.
88. The method of any one of claims 1-87, wherein after administering the population of pluripotent cells to the patient, the patient exhibits sustained disease remission.
89. The method of any one of claims 1-88, wherein after administering the population of pluripotent cells to the patient, the patient does not exhibit an angioedema attack for a period of from about two months to about one year.
90. The method of any one of claims 1-89, wherein after administering the population of pluripotent cells to the patient, the patient exhibits a serum concentration of C1-INH protein of at least about 7 mg/dl.
91. The method of claim 90, wherein after administering the population of pluripotent cells to the patient, the patient exhibits a serum concentration of C1-INH protein of from about 15 mg/dl to about 35 mg/dl.
92. The method of any one of claims 1-91, wherein after administering the population of pluripotent cells to the patient, the patient exhibits a serum concentration of C1-INH protein that is from about 40% to about 60% of a serum concentration of C1-INH protein exhibited by a subject that does not have HAE, optionally wherein the subject (i) is the same gender as the patient and/or (ii) has the same body mass index as the patient.
93. The method of any one of claims 1-92, wherein administration of the population of pluripotent cells to the patient reduces the patient's risk of suffocation due to laryngeal angioedema attacks.
94. A method of treating HAE in a patient in need thereof, the method comprising administering to the patient a lentiviral vector comprising a transgene that encodes a C1-INH protein.

95. A method of inducing sustained remission of HAE in a patient in need thereof, the method comprising administering to the patient a lentiviral vector comprising a transgene that encodes a C1-INH protein.

96. A method of preventing angioedema attacks in a patient diagnosed as having HAE, the method comprising administering to the patient a lentiviral vector comprising a transgene that encodes a C1-INH protein.

97. A method of reducing the risk of recurrent angioedema attacks in a patient diagnosed as having HAE, the method comprising administering to the patient a lentiviral vector comprising a transgene that encodes a C1-INH protein.

98. The method of claim 96 or 97, wherein the angioedema attacks occur in the patient's skin, mucosa, gastrointestinal tract, and/or genitourinary region.

99. A method of reducing the risk of developing laryngeal angioedema attacks in a patient diagnosed as having HAE, the method comprising administering to the patient a lentiviral vector comprising a transgene that encodes a C1-INH protein.

100. The method of any one of claims 94-99, wherein the lentiviral vector is administered systemically to the patient.

101. The method of claim 100, wherein the lentiviral vector is administered to the patient by way of intravenous injection.

102. The method of any one of claims 94-101, wherein the lentiviral vector comprises a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

103. The method of any one of claims 94-102, wherein the lentiviral vector is pseudotyped.

104. The method of claim 103, wherein the lentiviral vector comprises one or more envelope proteins from a virus selected from VSV, RD114 virus, MLV, FeLV, VEE, HFV, WDSV, SFV, Rabies virus, ALV, BIV, BLV, EBV, CAEV, SNV, ChTLV, STLV, MPMV, SMRV, RAV, FuSV, MH2, AEV, AMV, avian sarcoma virus CT10, and EIIV.

105. The method of claim 104, wherein the lentiviral vector comprises a VSV-G envelope protein.

106. The method of any one of claims 94-105, wherein the transgene is operably linked to a ubiquitous promoter.

107. The method of any one of claims 94-105, wherein the transgene is operably linked to a tissue-specific promoter.

108. The method of any one of claims 94-105, wherein the transgene is operably linked to a hepatocyte-specific promoter.

109. The method of any one of claims 94-105, wherein the transgene is operably linked to a transthyretin promoter, CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, EF1 α promoter, EFS promoter, PGK promoter, α -globin promoter, β -globin promoter, DC172 promoter, human serum albumin promoter, alpha1 antitrypsin promoter, thyroxine binding globulin promoter, or C1-INH promoter.

110. The method of any one of claims 94-109, wherein the transgene is operably linked to an enhancer.

111. The method of claim 110, wherein the enhancer comprises a β LCR.

112. The method of any one of claims 94-111, wherein the transgene is operably linked to a miRNA targeting sequence.

113. The method of claim 112, wherein the miRNA targeting sequence has complementarity to a miRNA that is endogenously expressed in a tissue in which expression of C1-INH is undesirable.

114. The method of any one of claims 94-113, wherein the patient is a mammal.

115. The method of claim 114, wherein the mammal is a human.

116. The method of any one of claims 94-115, wherein the patient has a loss-of-function mutation in an endogenous gene encoding C1-INH.

117. The method of claim 116, wherein the mutation is a deletion or substitution of an amino acid located within the RCL of C1-INH.

118. The method of claim 116, wherein the mutation is a deletion or substitution of K251.

119. The method of claim 116, wherein the mutation is selected from the group consisting of A436T, R444H, R444C, R444S, V432E, A443V, Y199TER, I462S, and R378C.

120. The method of any one of claims 94-119, wherein the patient has a mutation in an endogenous gene encoding C1-INH that causes (i) a deletion or (ii) expression of a truncated transcript.

121. The method of an one of claims 94-120, wherein the patient has a mutation in a gene encoding F12.

122. The method of any one of claims 116-121, wherein the mutation is heterozygous.

123. The method of any one of claims 116-121, wherein the mutation is homozygous.

124. The method of any one of claims 94-123, wherein the patient has previously been treated with one or more immunosuppressive agents, biologic agents, and/or corticosteroids.

125. The method of claim 124, wherein the patient has not responded to treatment with the one or more immunosuppressive agents, biologic agents, and/or corticosteroids.

126. The method of any one of claims 94-125, wherein the patient has previously been treated with one or more therapeutic agents selected from the group consisting of Berinert, Ruconest, Firazyr, and Kalbitor.

127. The method of claim 126, wherein the patient has not responded to treatment with the one or more therapeutic agents.

128. The method of any one of claims 94-127, wherein the patient has previously been treated with one or more prophylactic agents selected from the group consisting of Cinryze, Haegarda, Takhzyro, and an androgen.

129. The method of claim 128, wherein the patient has not responded to treatment with the one or more prophylactic agents.

130. The method of any one of claims 94-129, wherein the patient is less than 12 years old.

131. The method of claim 130, wherein the patient is less than 6 years old.

132. The method of any one of claims 94-129, wherein the patient is more than 6 years old.

133. The method of claim 132, wherein the patient is more than 12 years old.

134. The method of any one of claims 94-133, wherein prior to administering the lentiviral vector to the patient, the patient exhibits angioedema attacks with a frequency of from one to ten times per month.

135. The method of any one of claims 94-133, wherein prior to administering the lentiviral vector to the patient, the patient exhibits angioedema attacks with a frequency of one or two times per week.

136. The method of any one of claims 94-135, wherein after administering the lentiviral vector to the patient, the patient exhibits sustained disease remission.

137. The method of any one of claims 94-136, wherein after administering the lentiviral vector to the patient, the patient does not exhibit an angioedema attack for a period of from about two months to about one year.

138. The method of any one of claims 94-137, wherein after administering the lentiviral vector to the patient, the patient exhibits a serum concentration of C1-INH protein of at least about 7 mg/dl.

139. The method of claim 138, wherein after administering the lentiviral vector to the patient, the patient exhibits a serum concentration of C1-INH protein of from about 15 mg/dl to about 35 mg/dl.

140. The method of any one of claims 94-139, wherein after administering the lentiviral vector to the patient, the patient exhibits a serum concentration of C1-INH protein that is from about 40% to about 60% of a serum concentration of C1-INH protein exhibited by a subject that does not have HAE, optionally wherein the subject (i) is the same gender as the patient and/or (ii) has the same body mass index as the patient.

141. The method of any one of claims 94-140, wherein administration of the lentiviral vector to the patient reduces the patient's risk of suffocation due to laryngeal angioedema attacks.

142. A pharmaceutical composition comprising (i) a population of pluripotent cells comprising a transgene that encodes a C1-INH protein and (ii) one or more carriers, diluents, and/or excipients.

143. The pharmaceutical composition of claim 142, wherein the cells are human cells.

144. The pharmaceutical composition of claim 142 or 143, wherein the cells are HSCs or HPCs.

145. The pharmaceutical composition of claim 142 or 143, wherein the cells are embryonic stem cells.

146. The pharmaceutical composition of claim 142 or 143, wherein the cells are induced pluripotent stem cells.

147. The pharmaceutical composition of claim 142 or 143, wherein the cells are CD34+ cells.

148. The pharmaceutical composition of claim 147, wherein the CD34+ cells are myeloid progenitor cells.

149. The pharmaceutical composition of any one of claims 142-148, wherein the composition is formulated for administration to a human patient.

150. The pharmaceutical composition of claim 149, wherein the composition is formulated for intravenous injection to the human patient.

151. The pharmaceutical composition of claim 149 or 150, wherein the cells are autologous with respect to the patient.

152. The pharmaceutical composition of claim 149 or 150, wherein the cells are allogeneic with respect to the patient.

153. The pharmaceutical composition of claim 152, wherein the cells are HLA-matched to the patient.

154. The pharmaceutical composition of any one of claims 142-153, wherein the transgene is operably linked to a ubiquitous promoter.

155. The pharmaceutical composition of any one of claims 142-153, wherein the transgene is operably linked to a tissue-specific promoter.

156. The pharmaceutical composition of any one of claims 142-153, wherein the transgene is operably linked to a myeloid cell-specific promoter.

157. The pharmaceutical composition of any one of claims 142-153, wherein the transgene is operably linked to a CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, EF1 α promoter, EFS promoter, PGK promoter, α -globin promoter, β -globin promoter, DC172 promoter, human serum albumin promoter, alpha1 antitrypsin promoter, thyroxine binding globulin promoter, or C1-INH promoter.

158. The pharmaceutical composition of any one of claims 142-157, wherein the transgene is operably linked to an enhancer.

159. The pharmaceutical composition of claim 158, wherein the enhancer comprises a β LCR.

160. The pharmaceutical composition of any one of claims 142-159, wherein the transgene is operably linked to a miRNA targeting sequence

161. The pharmaceutical composition of claim 160, wherein the miRNA targeting sequence has complementarity to a miRNA that is endogenously expressed in a tissue in which expression of C1-INH is undesirable.

162. A pharmaceutical composition comprising (i) a lentiviral vector comprising a transgene that encodes a C1-INH protein and (ii) one or more carriers, diluents, and/or excipients.

163. The pharmaceutical composition of claim 162, wherein the lentiviral vector comprises a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

164. The pharmaceutical composition of claim 162 or 163, wherein the lentiviral vector is pseudotyped.

165. The pharmaceutical composition of claim 164, wherein the lentiviral vector comprises one or more envelope proteins from a virus selected from VSV, RD114 virus, MLV, FeLV, VEE, HFV, WDSV, SFV, Rabies virus, ALV, BIV, BLV, EBV, CAEV, SNV, ChTLV, STLV, MPMV, SMRV, RAV, FuSV, MH2, AEV, AMV, avian sarcoma virus CT10, and EIAV.

166. The pharmaceutical composition of claim 165, wherein the lentiviral vector comprises a VSV-G envelope protein.

167. The pharmaceutical composition of any one of claims 162-166, wherein the composition is formulated for administration to a human patient.

168. The pharmaceutical composition of claim 167, wherein the composition is formulated for intravenous injection to the human patient.

169. The pharmaceutical composition of any one of claims 162-168, wherein the transgene is operably linked to a ubiquitous promoter.

170. The pharmaceutical composition of any one of claims 162-168, wherein the transgene is operably linked to a tissue-specific promoter.

171. The pharmaceutical composition of any one of claims 162-168, wherein the transgene is operably linked to a hepatocyte-specific promoter.

172. The pharmaceutical composition of any one of claims 162-168, wherein the transgene is operably linked to a transthyretin promoter, CD11b promoter, sp146/p47 promoter, CD68 promoter, sp146/gp9 promoter, EF1 α promoter, EFS promoter, PGK promoter, α -globin promoter, β -globin promoter, DC172 promoter, human serum albumin promoter, alpha1 antitrypsin promoter, thyroxine binding globulin promoter, or C1-INH promoter.

173. The pharmaceutical composition of any one of claims 162-172, wherein the transgene is operably linked to an enhancer.

174. The pharmaceutical composition of claim 173, wherein the enhancer comprises a β LCR.

175. The pharmaceutical composition of any one of claims 162-174, wherein the transgene is operably linked to a miRNA targeting sequence

176. The pharmaceutical composition of claim 175, wherein the miRNA targeting sequence has complementarity to a miRNA that is endogenously expressed in a tissue in which expression of C1-INH is undesirable.

177. A kit comprising the pharmaceutical composition of any one of claims 142-176, wherein the kit further comprises a package insert instructing a user of the kit to administer the pharmaceutical composition to a human patient having HAE.

178. The kit of claim 177, wherein the package insert instructs a user of the kit to perform the method of any one of claims 1-141.