

NUCLEIC ACID OFF-SWITCHES AND METHODS AND USES THEREOF

TECHNICAL FIELD

This disclosure relates to methods of transiently modulating gene expression in a gene
5 therapy context, as well as vectors, nucleic acid, and related compositions that may be used in such methods.

BACKGROUND

Recombinant vectors derived from adeno-associated viruses (AAVs), among other gene
10 therapy modalities, have become a prevalent paradigm for heterologous gene expression due, at least in part, to the remarkable safety profile of this non-pathogenic virus, as well as its potential to achieve transgene expression in a variety of tissues. Intramuscularly administered AAV vectors have been explored as a vehicle for the delivery of various transgenes, including therapeutic and prophylactic proteins. While undergoing gene therapy with an AAV, it is sometimes desirable to
15 dampen the potency of transgene expression or to temporarily pause transgene expression for individuals who require care for a contraindication. Limited options are available for achieving tunable expression of a transgene following delivery with an AAV vector often due to risks of toxicity and off-target effects of inhibitors, as well as incompatible biodistribution and pharmacokinetic profiles of an AAV and an inhibitor. There remains a need for mechanisms for achieving tunable transgene
20 expression following gene therapy with an AAV or other gene delivery vehicle.

SUMMARY OF THE INVENTION

In one aspect, the disclosure features a method of reducing expression or activity of a protein of interest in a subject, the method including:

25 (a) administering to the subject a polynucleotide including (i) a transgene encoding the protein of interest and (ii) a binding site for an inhibitory RNA molecule; and subsequently

(b) administering to the subject an inhibitory RNA molecule, wherein the inhibitory RNA molecule has complementarity to the binding site, and wherein the inhibitory RNA molecule is administered following a desired period of time following step (a);

30 optionally wherein the polynucleotide is administered to the subject intramuscularly. In some embodiments, the polynucleotide is administered in the form of a viral vector (e.g., a recombinant adeno-associated viral vector (AAV) containing the same). In some embodiments, the polynucleotide is administered in the form of a non-viral formulation, such as a liposome, microvesicle, CRISPR system, or transposon system, among others.

35 In another aspect, the disclosure features a method of reducing expression or activity of a protein of interest in a subject, the method including administering to the subject an inhibitory RNA molecule, wherein the subject has previously been administered a polynucleotide including (i) a transgene encoding the protein of interest and (ii) a binding site having complementarity to the inhibitory RNA molecule, optionally wherein the polynucleotide is administered to the subject

intramuscularly. In some embodiments, the polynucleotide is administered in the form of a non-viral formulation, such as a liposome, microvesicle, CRISPR system, or transposon system, among others.

In another aspect, the disclosure features a method of temporally modulating expression or activity of a protein of interest in a subject, the method including:

5 (a) administering to the subject a polynucleotide including (i) a transgene encoding the protein of interest and (ii) a binding site for an inhibitory RNA molecule, wherein administration of the polynucleotide increases the expression or activity of the protein of interest in a subject; and subsequently

10 (b) administering to the subject an inhibitory RNA molecule, wherein the inhibitory RNA molecule has complementarity to the binding site, wherein the inhibitory RNA molecule is administered following a desired period of time following step (a), and wherein administration of the inhibitory RNA molecule reduces the expression or activity of the protein of interest in a subject. In some embodiments, the polynucleotide is administered to the subject intramuscularly. In some
15 embodiments, the polynucleotide is administered in the form of a non-viral formulation, such as a liposome, microvesicle, CRISPR system, or transposon system, among others.

In another aspect, the disclosure features a method of treating a disease in a subject in need thereof, the method including:

(a) administering to the subject a polynucleotide including (i) a transgene encoding a protein of interest and (ii) a binding site for an inhibitory RNA molecule; and
20 (b) administering to the subject an inhibitory RNA molecule, wherein the inhibitory RNA molecule has complementarity to the binding site, and wherein the inhibitory RNA molecule is administered following a desired period of time following step (a). In some embodiments, the polynucleotide is administered to the subject intramuscularly. In some embodiments, the polynucleotide is administered in the form of a non-viral formulation, such as a liposome,
25 microvesicle, CRISPR system, or transposon system, among others.

In another aspect, the disclosure features a method of treating a disease in a subject, the method including administering to the subject an inhibitory RNA molecule, wherein the subject has previously been administered a polynucleotide including (i) a transgene encoding the protein of interest and (ii) a binding site having complementarity to the inhibitory RNA molecule. In some
30 embodiments, the polynucleotide is administered to the subject intramuscularly. In some embodiments, the polynucleotide is administered in the form of a non-viral formulation, such as a liposome, microvesicle, CRISPR system, or transposon system, among others.

In some embodiments of any of the foregoing aspects or embodiments of the disclosure, the polynucleotide is administered to the subject in the form of a recombinant AAV containing the same.

35 In some embodiments, the recombinant AAV is administered to the subject between one and ten times (e.g., one, two, three, four, five, six, seven, eight, nine, or ten times). In some embodiments, the recombinant AAV is administered to the subject between one and five times (e.g., one, two, three, four, or five times). In some embodiments, the recombinant AAV is administered to the subject between three and five times (e.g., three, four, or five times). In some embodiments, the recombinant

AAV is administered to the subject between one and three times (e.g., one, two, or three times). In some embodiments, the recombinant AAV is administered to the subject once.

In some embodiments, the subject has or is at risk of developing a disease recited in Table 1.

In some embodiments, the transgene is a gene recited in Table 1.

5 In some embodiments, expression of the protein of interest is increased following administration of the recombinant AAV. In some embodiments, expression of the protein of interest is sustained for at least five days following administration of the recombinant AAV (e.g., from five days to ten years following administration of the recombinant AAV, such as from one month to five years following administration of the recombinant AAV, from one month to four years following
10 administration of the recombinant AAV, from one month to three years following administration of the recombinant AAV, from one month to three years following administration of the recombinant AAV, from one month to two years following administration of the recombinant AAV, or from one month to 12 months following administration of the recombinant AAV (e.g., one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months,
15 11 months, or 12 months following administration of the recombinant AAV).

In some embodiments, expression of the protein of interest is sustained for at least thirty days following administration of the recombinant AAV. In some embodiments, expression of the protein of interest is sustained for at least sixty days following administration of the recombinant AAV. In some
20 embodiments, expression of the protein of interest is sustained for at least ninety days following administration of the recombinant AAV. In some embodiments, expression of the protein of interest is sustained for at least 120 days following administration of the recombinant AAV.

In some embodiments, expression of the protein of interest is sustained for at least one year following administration of the recombinant AAV. In some embodiments, expression of the protein of interest is sustained between one to five years following administration of the recombinant AAV. In
25 some embodiments, expression of the protein of interest is sustained for at least five years, six years, seven years, eight years, nine years, ten years, or more than ten years following administration of the recombinant AAV.

In some embodiments, following administration of the recombinant AAV to the subject, the protein of interest is expressed by the subject at a therapeutic level or at a sub-therapeutic level.

30 In some embodiments, the inhibitory RNA molecule is selected from a small interfering RNA (siRNA), an antisense oligonucleotide (ASO), a double-stranded RNA (dsRNA), a short hairpin RNA (shRNA), a microRNA (miRNA), and a gapmer. In some embodiments, the inhibitory RNA molecule is an siRNA.

In some embodiments, the inhibitory RNA molecule further includes one or more
35 modifications selected from a nucleobase modification, a sugar modification, and an internucleoside linkage modification.

In some embodiments, the inhibitory RNA molecule further includes a hydrophobic moiety. In some embodiments, the hydrophobic moiety is a cholesterol, among other hydrophobic substituents described herein.

In some embodiments, the inhibitory RNA molecule is formulated within a delivery vehicle. In some embodiments, the delivery vehicle is a lipid-based carrier, a liposome, or a lipid nanoparticle.

In some embodiments, the inhibitory RNA molecule lacks sufficient complementarity to hybridize to an endogenous RNA sequence that occurs naturally in a cell of the subject. In some
5 embodiments, the inhibitory RNA molecule has less than 85% complementarity to an endogenous
RNA sequence that occurs naturally in a cell of the subject (e.g., less than 80% complementarity to an
endogenous RNA sequence that occurs naturally in a cell of the subject, less than 75%
complementarity to an endogenous RNA sequence that occurs naturally in a cell of the subject, less
10 than 70% complementarity to an endogenous RNA sequence that occurs naturally in a cell of the
subject, less than 65% complementarity to an endogenous RNA sequence that occurs naturally in a
cell of the subject, less than 60% complementarity to an endogenous RNA sequence that occurs
naturally in a cell of the subject, less than 55% complementarity to an endogenous RNA sequence
that occurs naturally in a cell of the subject, less than 50% complementarity to an endogenous RNA
15 sequence that occurs naturally in a cell of the subject, less than 45% complementarity to an
endogenous RNA sequence that occurs naturally in a cell of the subject, less than 40%
complementarity to an endogenous RNA sequence that occurs naturally in a cell of the subject, less
than 35% complementarity to an endogenous RNA sequence that occurs naturally in a cell of the
subject, less than 30% complementarity to an endogenous RNA sequence that occurs naturally in a
20 cell of the subject, less than 25% complementarity to an endogenous RNA sequence that occurs
naturally in a cell of the subject, less than 20% complementarity to an endogenous RNA sequence
that occurs naturally in a cell of the subject, less than 15% complementarity to an endogenous RNA
sequence that occurs naturally in a cell of the subject, or less than 10% complementarity to an
endogenous RNA sequence that occurs naturally in a cell of the subject).

In some embodiments, the inhibitory RNA molecule has complementarity to an mRNA
25 transcript or a portion thereof that is transcribed from the recombinant AAV vector, wherein the mRNA
transcript encodes the protein of interest.

In some embodiments, the inhibitory RNA molecule has at least 50% complementarity to the
inhibitory RNA molecule binding site (e.g., at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%,
96%, 97%, 98%, 99%, or 100% complementarity) to the inhibitory RNA molecule binding site. In some
30 embodiments, the inhibitory RNA molecule has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,
93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementarity to the inhibitory RNA molecule
binding site.

In some embodiments, the inhibitory RNA molecule is 100% complementary to a sequence of
at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least
35 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at
least 27, at least 28, at least 29, or at least 30 contiguous nucleotides within the inhibitory RNA
molecule binding site.

In some embodiments, the inhibitory RNA molecule binding site is present within one or more
of the following locations relative to the transgene: the 3' untranslated region (UTR), the 5' UTR, a

region upstream of the start codon, a region downstream of the stop codon, or a region in the open reading frame of the transgene.

In some embodiments, the inhibitory RNA molecule is intramuscularly administered to the subject, among other routes of administration described herein.

5 In some embodiments, administration of the inhibitory RNA molecule reduces expression of the protein of interest by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%, as compared to expression prior to administration of the inhibitory RNA molecule. In some embodiments, administration of the inhibitory RNA molecule reduces the expression of the protein of interest by about 2-fold, by about 3-fold, by about 4-fold, by
10 about 5-fold, by about 6-fold, by about 7-fold, by about 8-fold, by about 9-fold, by about 10-fold, by about 15-fold, by about 20-fold, by about 25-fold, by about 30-fold, by about 35-fold, by about 40-fold, by about 45-fold, by about 50-fold, or greater than 50-fold, as compared to expression prior to administration of the inhibitory RNA molecule.

 In some embodiments, administration of the inhibitory RNA molecule reduces expression of
15 the protein of interest for at least five days. In some embodiments, administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least thirty days. In some embodiments, administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least sixty days. In some embodiments, administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least ninety days. In some embodiments,
20 administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least 120 days.

 In some embodiments, administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least one year. In some embodiments, administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least five years. In some
25 embodiments, administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least 5, 6, 7, 8, 9, 10, or more than 10 years.

 In some embodiments, the subject is a human.

 In another aspect, the disclosure features a pharmaceutical composition including the polynucleotide of any one of the preceding embodiments and a pharmaceutically acceptable
30 excipient, carrier, or diluent. In some embodiments, the polynucleotide is incorporated into a viral vector (e.g., a recombinant AAV vector), into a liposome, or into a microvesicle. In some embodiments, the polynucleotide is provided as a component of a CRISPR system or as a component of a transposon system.

 In some embodiments, the AAV vector is a serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, rh10, or rh74 AAV
35 vector. In some embodiments, the AAV vector is an AAV8 or AAV9 serotype vector. In some embodiments, the AAV vector contains one or more synthetic capsid proteins.

 In another aspect, the disclosure features a pharmaceutical composition including the inhibitory RNA molecule of any one of the preceding embodiments and a pharmaceutically acceptable excipient, carrier, or diluent.

In another aspect, the disclosure features a kit including a package insert that instructs a user to perform any one of the preceding methods. In some embodiments, the kit further includes the pharmaceutical composition including the inhibitory RNA molecule of any one of the preceding embodiments and a pharmaceutically acceptable excipient, carrier, or diluent or the pharmaceutical composition including the polynucleotide of any one of the preceding embodiments and a pharmaceutically acceptable excipient, carrier, or diluent. In some embodiments, the kit further includes one or more binding molecules to detect the expression or activity of the protein of interest.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are included to illustrate embodiments of the disclosure and further an understanding of its implementations.

FIG. 1 is a diagram of an exemplary adeno-associated viral (AAV) vector transgene, showing inverted terminal repeats (ITRs) of the AAV genome that flank the expression construct of the cyno dulaglutide transgene (CyDula) and a region in the 3' untranslated region (UTR) that serves as a target site for small inhibitory RNA (siRNA) molecules.

FIG. 2 is a schematic of the experimental design for monitoring transgene expression levels in mice. At day 0 (d0), mice were intramuscularly administered an AAV9 vector containing the CyDula transgene. At day 13 (d13), a blood sample was obtained from the mice to establish baseline expression levels of the CyDula transgene. At day 14 (d14), mice were intramuscularly administered an siRNA or appropriate controls. Every two weeks following administration of the siRNA, additional blood samples were obtained to monitor potential changes in transgene expression levels over time.

FIGS. 3A-3B are graphs plotting ELISA data that show measured serum protein concentrations of CyDula (**FIG. 3A**) or relative serum protein concentrations of CyDula as compared to the average day 13 serum expression level for each group (**FIG. 3B**) to evaluate potential adverse effects on gene expression following intramuscular administration of scrambled siRNA negative control molecules, in which one was modified to contain a hydrophobic moiety (C1-HM), and the other was unmodified (C1).

FIG. 4 is a graph of ELISA data showing measured serum protein concentrations of CyDula following intramuscular administration of equal doses of sequence-equivalent siRNA molecules, in which one siRNA molecule was modified to contain a hydrophobic moiety (T1-HM), and the other was an unmodified siRNA (T1).

FIG. 5 is a graph depicting serum protein concentrations of CyDula (relative to the day 13 group average serum CyDula expression level) over time following intramuscular administration of 200 µg of the hydrophobic moiety-modified T1-HM siRNA molecules, relative to a PBS-injected control.

FIG. 6 is a graph depicting serum protein concentrations of CyDula (relative to the day 13 group average serum CyDula expression level) over time following intramuscular administration of 50 µg of the hydrophobic moiety-modified T1-HM siRNA molecules, relative to a PBS-injected control.

FIG. 7 is a graph depicting serum protein concentrations of CyDula at day 28 relative to the day 13 CyDula expression level following varying doses (6 µg, 12 µg, 25 µg, 50 µg, 100 µg, 200 µg, or 400 µg) of siRNA-1 relative to the same dose of a non-targeting siRNA.

FIG. 8 is a bar graph depicting serum protein concentrations of CyDula two weeks after intramuscular administration of the indicated siRNA molecules and doses relative to a PBS-administered control.

FIG. 9 is a bar graph depicting mRNA transcript levels of CyDula in muscle tissue measured two weeks after intramuscular administration of the indicated siRNA molecules and doses relative to a PBS-administered control.

FIG. 10 is a dose response curve showing relative serum protein concentrations of CyDula two weeks after intramuscular administration of siRNA-1 at the indicated doses, as compared to the relative serum concentrations prior to siRNA administration.

FIG. 11 is a dose response curve showing mRNA transcript levels of CyDula in muscle tissue measured two weeks after intramuscular administration of siRNA-1 at the indicated doses, as compared to the relative serum concentrations prior to siRNA administration.

DEFINITIONS

Unless otherwise defined herein, scientific, and technical terms used herein have the meanings that are commonly understood by those of ordinary skill in the art. In the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The use of "or" means "and/or" unless stated otherwise. The use of the term "including," as well as other forms, such as "includes" and "included," is not limiting.

As used herein, the term "about," as applied to one or more values of interest, refers to a value that falls within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of a stated reference value, unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

As used herein, the term "comprise" or variations thereof (e.g., comprises or comprising) will be understood to include a feature or a plurality of features but not exclude additional features or plurality of features.

As used herein in the context of a therapeutic intervention and a protein of interest, the term "contraindicated" refers to instances in which administration of the therapeutic intervention (e.g., a small molecule, biologic drug product, cell therapy, gene therapy, surgical procedure, dietary supplement, or other therapeutic agent intended to produce a beneficial effect in a patient) would either negate the effect of, or produce toxicity as a result of, expression of the protein of interest. Exemplary contraindications that may be encountered by a patient undergoing treatment with the compositions and methods of the disclosure are described herein. In such instances, a patient may be administered an inhibitory RNA molecule to transiently suppress expression of the protein of interest, at least until such a time as the therapeutic intervention has ceased.

As used herein, the terms "polynucleotide" or "nucleic acid" refer to polymers of nucleotides of

any length and include DNA and/or RNA. The nucleotides may be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction. Thus, for instance, polynucleotides as defined herein include, without limitation, single- and double-stranded DNA, DNA including single- and double-stranded regions, single- and double-stranded RNA, and RNA including single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or include single- and double-stranded regions. In addition, the term "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. The term "polynucleotide" specifically includes complementary DNA (cDNA).

A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after synthesis, such as by conjugation with a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally-occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, and the like) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, and the like), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, and the like), those with intercalators (e.g., acridine, psoralen, and the like), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, and the like), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro-, or 2'-azido-ribose, carbocyclic sugar analogs, α -anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), "(O)NR₂" ("amidate"), P(O)R, P(O)OR', CO or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl,

cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

As used herein, the terms "adenine" and "adenosine" are interchangeable terms in reference to a nucleotide that has an adenine base. As used herein, the terms "cytosine" and "cytidine" are interchangeable terms in reference to a nucleotide that has a cytosine base. As used herein, the terms "guanine" and "guanine" are interchangeable terms in reference to a nucleotide that has a guanine base. As used herein, the terms "thymine" and "thymidine" are interchangeable terms in reference to a nucleotide that has a thymine base. As used herein, the term "uracil" and "uridine" are interchangeable terms in reference to a nucleotide that has a uracil base.

As used herein, the term "nucleoside" refers to a molecule made up of a heterocyclic base and its sugar.

As used herein, the term "nucleotide" refers to a nucleoside having a phosphate group, or a variant thereof, on its 3' or 5' sugar hydroxyl group. Examples of phosphate group variants include, but are not limited to, saturated alkyl phosphonates, unsaturated alkenyl phosphonates, phosphorothioates, and phosphoramidites.

In the context of this disclosure, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring (e.g., modified) portions that function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

As used herein, a "coding sequence" refers to an open reading frame (ORF) in a nucleic acid that, upon expression, yields a polypeptide or protein. An ORF is a continuous stretch of DNA or RNA beginning with a start codon (e.g., methionine (ATG or AUG)) and ending with a stop codon (e.g., TAA, TAG or TGA, or UAA, UAG or UGA). An ORF typically encodes a protein. It will be understood that the sequences disclosed herein may further include additional elements, e.g., 5' and 3' UTRs, but that those elements, unlike the ORF, need not necessarily be present in an RNA polynucleotide (e.g., an mRNA transcript) disclosed herein.

As used herein, "messenger RNA," "mRNA," or "mRNA transcript" is any RNA molecule that encodes a (at least one) protein (e.g., a polypeptide) or fragment thereof and can be translated to produce the encoded protein or the fragment thereof *in vitro*, *in vivo*, *in situ*, or *ex vivo*. Structural and topological features as well as post-transcriptional modifications of mRNA are described herein and are well-known in the art.

As used herein, the term "inhibitory nucleic acid" refers to a nucleic acid that comprises a guide strand sequence that hybridizes to at least a portion of a target nucleic acid, e.g., ATXN2 RNA, mRNA, pre-mRNA, or mature mRNA, and inhibits its expression or activity. An inhibitory nucleic acid may target a protein coding region (e.g., exon) or non-coding region (e.g., 5'UTR, 3'UTR, intron, etc.) of a target nucleic acid. In some embodiments, an inhibitory nucleic acid is a single stranded or double stranded molecule. An inhibitory nucleic acid may further comprise a passenger strand

sequence on a separate strand (e.g., double stranded duplex) or in the same strand (e.g., single stranded, self-annealing duplex structure). In some embodiments, an inhibitory nucleic acid is an interfering RNA molecule, such as short interfering RNA (siRNA), double-stranded RNA (dsRNA), microRNA (miRNA), antisense oligonucleotide (ASO), or gapmer.

5 As used herein, the term "siRNA" refers to small interfering RNA duplexes that induce the RNA interference (RNAi) pathway. siRNA molecules may vary in length (generally, between 10 and 30 base pairs) and may contain varying degrees of complementarity to their target mRNA. The term "siRNA" includes duplexes of two separate strands, as well as single strands that optionally form hairpin structures including a duplex region.

10 As used herein, the term "antisense strand" refers to the strand of the siRNA duplex that contains some degree of complementarity to the target gene.

 As used herein, the term "sense strand" refers to the strand of the siRNA duplex that contains complementarity to the antisense strand.

 The term "interfering RNA molecule" refers to an RNA molecule, such as a small interfering
15 RNA (siRNA), microRNA (miRNA), short hairpin RNA (shRNA), or an antisense oligonucleotide (ASO) that suppresses the endogenous function of a target RNA transcript.

 As used herein, the terms "complementary," "complementarity," or variations thereof (e.g., "having complementarity" or "has complementarity") refer to two nucleotides that form canonical Watson-Crick base pairs. For the avoidance of doubt, Watson-Crick base pairs in the context of the
20 present disclosure include adenine-thymine, adenine-uracil, and cytosine-guanine base pairs. A proper Watson-Crick base pair is referred to in this context as a "match," while each unpaired nucleotide, and each incorrectly paired nucleotide, is referred to as a "mismatch." Alignment for purposes of determining percent nucleic acid sequence complementarity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available
25 computer software such as BLAST, BLAST-2, or Megalign software.

 As used herein, the term "percent (%) sequence complementarity" with respect to a reference polynucleotide sequence is defined as the percentage of nucleic acids in a candidate sequence that are complementary to the nucleic acids in the reference polynucleotide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence
30 complementarity. A given nucleotide is considered to be "complementary" to a reference nucleotide as described herein if the two nucleotides form canonical Watson-Crick base pairs. For the avoidance of doubt, Watson-Crick base pairs in the context of the present disclosure include adenine-thymine, adenine-uracil, and cytosine-guanine base pairs. A proper Watson-Crick base pair is referred to in this context as a "match," while each unpaired nucleotide, and each incorrectly paired
35 nucleotide, is referred to as a "mismatch." Alignment for purposes of determining percent nucleic acid sequence complementarity 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 complementarity over the
40 full length of the sequences being compared. As an illustration, the percent sequence

complementarity of a given nucleic acid sequence, A, to a given nucleic acid sequence, B, (which can alternatively be phrased as a given nucleic acid sequence, A that has a certain percent complementarity to a given nucleic acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

- 5 where X is the number of complementary base pairs in an alignment (e.g., as executed by computer software, such as 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 sequence A is not equal to the length of nucleic acid sequence B, the percent sequence complementarity of A to B will not equal the percent sequence complementarity of B to A. As used herein, a query nucleic acid
10 sequence is considered to be "completely complementary" to a reference nucleic acid sequence if the query nucleic acid sequence has 100% sequence complementarity to the reference nucleic acid sequence.

As used herein, "peptide" is less than or equal to 50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

- 15 As used herein, "polypeptide" refers to a polymer of amino acid residues linked together by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. Typically, however, a polypeptide will be at least 50 amino acids and the polypeptide is termed a peptide. If the polypeptide is a peptide, it will be about 5 amino acid residues long. Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic
20 polypeptides, homologs, orthologs, paralogs, fragments, and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, a trimer, or a tetramer. The term polypeptide may also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analog of a corresponding naturally occurring amino acid.

- 25 As used herein, the terms "protein" refers to contiguous amino acids or amino acid residues. Typically, proteins have a function. However, proteins also encompass polypeptides and smaller contiguous amino acid sequences that do not have a functional activity. The functional proteins of this invention include, but are not limited to, enzymes, dehydrogenases, hydrolases, oxidoreductases, transferases, lyases, ligases, receptors, receptor ligands, cytokines, antibodies, immunomodulatory
30 molecules, signaling molecules, or proteins that are tagged or modified (e.g., for diagnostic or other clinical applications). Useful general classes of enzymes include, but are not limited to, proteases, cellulases, lipases, hemicellulases, laccases, amylases, glucoamylases, esterases, lactases, polygalacturonases, galactosidases, ligninases, oxidases, peroxidases, glucose isomerases, nitrilases, hydroxylases, polymerases and depolymerases. In addition to enzymes, the encoded
35 proteins which can be used in this invention include, but are not limited to, transcription factors, antibodies, receptors, growth factors (any of the PDGFs, EGFs, FGFs, SCF, HGF, TGFs, TNFs, insulin, IGFs, LIFs, oncostatins, and CSFs), immunomodulators, peptide hormones, cytokines, integrins, interleukins, adhesion molecules, thrombomodulatory molecules, protease inhibitors, angiostatins, defensins, cluster of differentiation antigens, interferons, chemokines, antigens including
40 those from infectious viruses and organisms, oncogene products, thrombopoietin, erythropoietin,

tissue plasminogen activator, and any other biologically active protein which is desired for use in a clinical setting. Such proteins are well known in the art. Also included are deletion mutants of such proteins (e.g., in which an individual residue or a plurality of residues are deleted from the sequence), individual domains of such proteins, fusion proteins made from such proteins, and mixtures of such proteins.

As used herein, the term "antibody" refers to a molecule that specifically binds to, or is immunologically reactive with, a particular antigen and includes at least the variable domain of a heavy chain, and normally includes at least the variable domains of a heavy chain and of a light chain of an immunoglobulin. Antibodies and antigen-binding fragments, variants, or derivatives thereof include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, e.g., Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a V_L or V_H domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies. Antibody molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Moreover, unless otherwise indicated, the term "monoclonal antibody" (mAb) is meant to include both intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')₂ fragments) that are capable of specifically binding to a target protein. Fab and F(ab')₂ fragments lack the Fc fragment of an intact antibody.

The term "antigen-binding fragment," as used herein, refers to one or more fragments of an immunoglobulin that retain the ability to specifically bind to a target antigen. The antigen-binding function of an immunoglobulin can be performed by fragments of a full-length antibody. The antibody fragments can be a Fab, F(ab')₂, scFv, SMIP, diabody, a triabody, an affibody, a nanobody, an aptamer, or a domain antibody. Examples of binding fragments encompassed by the term "antigen-binding fragment" of an antibody include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L, and C_H1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb (Ward et al., Nature 341:544-546, 1989) including V_H and V_L domains; (vi) a dAb fragment that consists of a V_H domain; (vii) a dAb that consists of a V_H or a V_L domain; (viii) an isolated complementarity determining region (CDR); and (ix) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv)). These antibody fragments can be obtained using conventional techniques known to those of skill in the art, and the fragments can be screened for utility in the same manner as intact antibodies. Antigen-binding fragments can be produced by recombinant DNA techniques, enzymatic or chemical cleavage of intact immunoglobulins, or, in certain cases, by chemical peptide synthesis procedures known in the art.

As used herein, "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:

$$100 \text{ multiplied by (the fraction } X/Y \text{)}$$

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 "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. Certain vectors that can be used for the expression of transgenes as described herein include plasmids that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Other useful vectors for expression of a transgenes contain polynucleotide sequences that enhance the rate of translation of these genes or improve the stability or nuclear export of the mRNA that results from gene transcription. These sequence elements include, e.g., 5' and 3' untranslated regions, an internal ribosomal entry site (IRES), and polyadenylation signal site in order to direct efficient transcription of the gene carried on the expression vector. The 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.

As used herein, the terms “adeno-associated virus” and “AAV” include, but are not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, and any other AAV now known or later discovered. See, e.g., Fields et al. *Virology*, 4th ed. Lippincott-Raven Publishers, Philadelphia, 1996. Additional AAV serotypes and clades have been identified recently. (See, e.g., Gao et al. *J. Virol.* 78:6381 (2004); Moris et al. *Viol.* 33:375 (2004). The genomic sequences of various serotypes of AAV, as well as the sequences of the native inverted terminal repeats (ITRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC—002077, NC—001401, NC—001729, NC—001863, NC—001829, NC—001862, NC—000883, NC—001701, NC—001510, NC—006152, NC—006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, AY631966, AX753250, EU285562, NC—001358, NC—001540, AF513851, AF513852 and AY530579; the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences. See also, e.g., Bantel-Schaal et al. *J. Virol.* 73:939 (1999); Chiorini et al. *J. Virol.* 71:6823 (1997); Chiorini et al. *J. Virol.* 73:1309 (1999); Gao et al. *Proc. Nat. Acad. Sci. USA* 99:11854 (2002); Moris et al. *Viol.* 33:375 (2004); Muramatsu et al. *Viol.* 221:208 (1996); Ruffing et al. *J. Gen. Virol.* 75:3385 (1994); Rutledge et al. *J. Virol.* 72:309 (1998); Schmidt et al. *J. Virol.* 82:8911 (2008); Shade et al. *J. Virol.* 58:921 (1986); Srivastava et al. *J. Virol.* 45:555 (1983); Xiao et al. *J. Virol.* 73:3994 (1999); WO 00/28061, WO 99/61601, WO 98/11244; and US 6,156,303; the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences.

From a structural perspective, AAV is a nonpathogenic parvovirus composed of a 4.7 kb single- stranded DNA genome within a non-enveloped, icosahedral capsid. The genome contains three open reading frames (ORF) flanked by inverted terminal repeats (ITR) that function as the viral origin of replication and packaging signal. The Rep ORF encodes four nonstructural proteins that play roles in viral replication, transcriptional regulation, site-specific integration, and virion assembly. The Cap ORF encodes three structural proteins (VP 1-3) that assemble to form a 60-mer viral capsid. Finally, an ORF present as an alternate reading frame within the Cap gene produces the assembly-activating protein (AAP), a viral protein that localizes AAV capsid proteins to the nucleolus and functions in the capsid assembly process. There are several naturally occurring (“wild-type”) serotypes and over 100 known variants of AAV, each of which differs in amino acid sequence, particularly within the hypervariable regions of the capsid proteins, and thus in their gene delivery properties. No AAV has been associated with any human disease, making recombinant AAV attractive for clinical applications.

The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits, are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC_002077.1 (AAV1), AF063497.1 (AAV1), NC_001401.2 (AAV2), AF043303.1 (AAV2),

J01901.1 (AAV2), U48704.1 (AAV3A), NC_001729.1 (AAV3A), AF028705.1 (AAV3B), NC_001829.1 (AAV4), U89790.1 (AAV4), NC_006152.1 (AA5), AF085716.1 (AAV-5), AF028704.1 (AAV6), NC_006260.1 (AAV7), AF513851.1 (AAV7), AF513852.1 (AAV8), NC_006261.1 (AAV-8), AY530579.1 (AAV9), AAT46337 (AAV10) and AAO88208 (AAVrh10); the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences. See also, e.g., Srivastava et al. (1983) J. Virology 45:555; Chiorini et al. (1998) J. Virology 71:6823; Chiorini et al. (1999) J. Virology 73: 1309; Bantel-Schaal et al. (1999) J. Virology 73:939; Xiao et al. (1999) J. Virology 73:3994; Muramatsu et al. (1996) Virology 221:208; Shade et al. (1986) J. Virol. 58:921; Gao et al. (2002) Proc. Nat. Acad. Sci. USA 99: 11854; Moris et al. (2004) Virology 33:375-383; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Pat. No. 6,156,303.

As used herein, the term “transgene” refers to a recombinant nucleic acid (e.g., DNA or cDNA) encoding a gene product (e.g., a therapeutic gene product). The gene product may be an RNA, peptide, polypeptide, or protein. In addition to the coding region for the gene product, the transgene may 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 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 functional elements.

As used herein, the terms “wild-type,” “naturally occurring,” or “non-mutant” form of a gene refers to a nucleic acid that encodes a protein associated with normal or non-pathogenic activity (e.g., a protein lacking a mutation). In some embodiments, the wildtype gene may serve as a reference to compare a variant gene that is associated with a genetic disorder, e.g., as described in Table 1 herein.

As used herein, the term “variant” or “mutant” refers to any gene with a change in sequence, such that the sequence is not identical to that of the wild-type gene and results in an altered form of the gene. A mutation may be selected from the group including a single nucleotide point mutation that results in a premature termination codon, a single nucleotide insertion, a single nucleotide deletion, the insertion of two or more contiguous nucleotides, the deletion of two or more contiguous nucleotides, the duplication of a contiguous region within a gene (e.g., an exon), or the deletion of a contiguous region within a gene. A mutated gene may include a single mutation, or multiple mutations. A mutation may occur in any region of the gene. Gene mutations include the substitution, insertion, or deletion of a single base in DNA or the substitution, insertion, deletion, or rearrangement of multiple bases or larger sections of genes or chromosomes, including repeat expansions.

As used herein, the terms “hybridization,” “annealing,” or “binding” of nucleic acids is achieved when one or more nucleoside residues within a polynucleotide base pairs with one or more complementary nucleosides to form a stable duplex. The base pairing is typically driven by hydrogen bonding events. Hybridization includes Watson-Crick base pairs formed from natural and/or modified nucleobases. The hybridization can also include non-Watson-Crick base pairs, such as wobble base pairs (guanosine-uracil, hypoxanthine-uracil, hypoxanthine-adenine, and hypoxanthine-cytosine) and

Hoogsteen base pairs. Nucleic acids need not be 100% complementary to undergo hybridization. For example, one nucleic acid may be, e.g., 95% complementary, 90%, complementary, 85% complementary, 80% complementary, 75% complementary, 70% complementary, 65% complementary, 60% complementary, 55% complementary, 50% complementary, or less, relative to another nucleic acid, but the two nucleic acids may still form sufficient base pairs with one another so as to hybridize.

As used herein, the phrase "operably linked" refers to a functional connection between two or more molecules, constructs, transcripts, entities, moieties, or the like. For example, one segment of DNA may be operably linked to another segment of DNA if they are positioned relative to one another on the same contiguous DNA molecule and have a structural or functional relationship, such as a promoter or enhancer that is positioned relative to a coding region so as to facilitate transcription of the coding region. In other examples, the operably linked nucleic acids are not contiguous, but are positioned in such a way that they have a functional relationship with each other as nucleic acids or as proteins that are expressed by them. Enhancers, for example, do not have to be contiguous. Linking may be accomplished by ligation at convenient restriction sites or by using synthetic oligonucleotide adaptors or linkers.

As used herein, the term "contacting" (i.e., contacting a cell with an agent) is intended to include incubating the agent and the cell together *in vitro* (e.g., adding the agent to cells in culture) or administering the agent to a subject such that the agent and cells of the subject are contacted *in vivo*. The term "contacting" is not intended to include exposure of cells to an agent that may occur naturally in a subject (i.e., exposure that may occur as a result of a natural physiological process).

As used herein, the terms "associated with," "conjugated," "linked," "attached," and "tethered," when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, e.g., physiological conditions. An "association" need not be strictly through direct covalent chemical bonding. It may also suggest ionic or hydrogen bonding or a hybridization-based connectivity sufficiently stable such that the "associated" entities remain physically associated.

As used herein "modified" refers to a changed state or structure of a molecule (e.g., a polynucleotide; e.g., DNA or mRNA; e.g., a polypeptide or protein; e.g., an amino acid residue) of the invention. Molecules may be modified in many ways, such as structural modifications (e.g., mutation of one or more base pairs or amino acid residues) or chemical modifications (e.g., methylation, acetylation, reduction or oxidation, glycosylation, lipidation, ubiquitination, of one or more base pairs or amino acid residues). In some embodiments, a molecule such as a DNA or an mRNA is modified to remove, reduce, or eliminate DRACH motifs to reduce the number of m6A methylation modifications in a gene or coding sequence of interest.

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. Expression of a gene of interest in a subject can manifest, for example, by detecting: an increase in the quantity or concentration of mRNA encoding a corresponding protein (as assessed, e.g., using RNA detection procedures such as quantitative polymerase chain reaction (qPCR) and RNA sequencing (RNA-seq) techniques, among other RNA detection methods known in the art), an increase in the quantity or concentration of a corresponding protein (as assessed, e.g., using protein detection methods described herein or known in the art, such as enzyme-linked immunosorbent assays (ELISA), Western blot, or mass spectrometry, among others), and/or an increase in the activity of a corresponding protein (e.g., in the case of an enzyme, as assessed using an enzymatic activity assay known in the art) in a sample obtained from the subject.

As used herein, the term "stability" in reference to biological material or a molecule (e.g., a polynucleotide or a polypeptide) refers to the balance of production (e.g., transcription or translation) and decay or degradation or the steady-state levels of the biological material in a system, such as a whole organism, an organ, a tissue or subset of tissues, a cell or a subset of cells, or in a dish or receptacle. In some embodiments, stability refers to the half-life of the biological material or molecule.

As used herein, the term "reduce," with respect to expression of a gene (i.e., expression levels), refers to decreasing (e.g., suppressing, lessening, minimizing, diminishing, or dampening) the rate, degree, or magnitude of any one or more aspects of gene expression (e.g., transcription, RNA processing, translation, post-translational modifications, and/or protein activity (e.g., protein function or catalytic activity)). Reduction of gene expression (e.g., expression of a transgene encoding a protein of interest; e.g., a transgene in a recombinant AAV vector, as described herein) may be induced by an exogenous agent, such as an inhibitory RNA as described herein. The degree and/or length of time by which gene expression is reduced may depend on physicochemical properties of the one or more inhibitory RNA molecules including the physicochemical properties of a delivery vehicle, the frequency of administration, the route of administration, and/or the amount administered. Gene expression levels may be reduced by about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or by about 100% relative to expression levels of one or more reference samples such as, e.g., a sample obtained prior to administration of an inhibitory RNA molecule. Reduction of gene expression may be confirmed or quantified by any suitable method for measuring expression of a nucleic acid or a protein (including protein activity) known in the art or described herein, in which a sample in which gene expression is reduced is compared to an equivalent reference sample (e.g., a sample obtained prior to administration of an inhibitory RNA molecule).

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 therefrom.

As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, in a Petri dish, etc., rather than within an organism (e.g., animal, plant, or microbe).

5 As used herein, the term "*in vivo*" refers to events that occur within an organism (e.g., animal, plant, or microbe or cell or tissue thereof).

As used herein, the term "*ex vivo*" refers to events that occur to a component of an organism (e.g., a tissue, a cell, or a subcellular fraction) when it is taken from the natural environment (e.g., from the body or natural structure) and placed into an artificial environment (e.g., a test tube or a culture dish, flask, or other receptacle) for experimental or clinical applications. In some instances, *ex vivo* experimentation or applications may involve administering (e.g., implanting, injecting, depositing, infusing, among other suitable routes of administration) the component to the same subject or a separate recipient subject following one or more *ex vivo* applications.

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As used herein, "treatment" and "treating" in reference to a disease or condition, 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 or symptoms 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 have or at risk of having the condition or disorder (e.g., for prophylactic treatment).

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As used herein, the term "sample" refers to a subset of its tissues, cells or component parts (e.g. body fluids, including but not limited to peripheral blood, serum, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, bronchoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, and umbilical cord blood). A sample further may include a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. A sample further refers to a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecules.

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As used herein, the term “reference level” herein refers to a value from a “reference sample” or a “control sample” to determine the effect induced by the methods described herein. A reference level may be a metric or measurement determined prior to administration or implementation of the method (e.g., one or more codon optimization methods or one or more methods of treatment described herein). A reference level may be a metric or measurement determined in a reference sample in which the methods described herein were not administered (e.g., a negative control sample; e.g., a healthy subject control or a subject with a disease or condition). A reference level may be a metric or measurement determined in a reference sample that exhibits a known or expected effect in order to evaluate the efficacy of the methods described herein (e.g., effects produced by routine methods of protein production or effects produced by known methods of treatment). In some embodiments, a reference level may be a predetermined value or a value. As the skilled artisan will appreciate, the reference level is predetermined and set to meet the requirements in terms of, for example, specificity and/or sensitivity. It may be, for example, that assay sensitivity or specificity, respectively, has to be set to certain limits, e.g., 80%, 90% or 95%. These requirements may also be defined in terms of positive or negative predictive values. In one embodiment, the reference level is determined in healthy individuals. The reference value in one embodiment has been predetermined in the disease entity to which a subject belongs. In certain embodiments, the reference level can be set to any percentage between, e.g., 25% and 75% of the overall distribution of the values in a disease entity investigated. In other embodiments, the reference level can be set to, for example, the median, tertiles, quartiles, or quintiles as determined from the overall distribution of the values in a disease entity investigated or in a given population. In one embodiment, the reference level is set to the median value as determined from the overall distribution of the values in a disease entity investigated. In some embodiments, the reference level may depend on the sex of the patient, e.g., males may have a different reference level than females.

As used herein, the terms “effective amount,” “therapeutically effective amount,” and a “sufficient amount” of a composition described herein refer to a quantity sufficient to, when administered to the subject (e.g., a mammal; e.g., a human subject) yield beneficial or desired results, including clinical results, and, as such, an “effective amount” or synonym thereto depends upon the context in which it is being applied. The amount of a given composition described herein that will correspond to such an amount will vary depending upon an assortment of factors, such as the given therapeutic agent (e.g., a polynucleotide or a vector; e.g., an AAV comprising a transgene as described herein or a plurality thereof, an inhibitory RNA or a plurality thereof), the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, weight) or host being treated, and the like, but can nevertheless be routinely determined by one skilled in the art. Also, as used herein, a “therapeutically effective amount” of a composition of the present disclosure is an amount which results in a beneficial or desired result in a subject as compared to a reference (e.g., the subject prior to treatment, a healthy control, or an untreated subject). As defined herein, a therapeutically effective amount of a composition of the present disclosure may be readily determined by one of ordinary skill by routine methods known in the art.

As used herein, the term “therapeutic level,” in reference to protein expression, refers to a protein expression level that is within an acceptable range to confer a desired effect from a treatment. A therapeutic level may refer to a physiologically acceptable expression level based on a healthy control subject or a median expression level based on a plurality of healthy control subjects. A therapeutic level may refer to an expression level that falls within a range of accepted expression levels based on subject characteristics such as, e.g., a subject’s age, sex, weight, height, ethnicity, lifestyle, and/or one or more comorbidities. A therapeutic level may refer to an expression level that exceeds physiological values, such that the therapeutic expression level has about a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 425%, 450%, 475%, 500%, or more than about 500% greater relative to an expression level based on a healthy control subject or a median expression level based on a plurality of healthy control subjects.

As used herein, the term “sub-therapeutic level,” in reference to protein expression, refers to a protein expression level that is below a therapeutic level. A sub-therapeutic level of protein expression may refer to an expression level below a physiologically acceptable expression level based on a healthy control subject or a median expression level based on a plurality of healthy control subjects. A sub-therapeutic level may refer to an expression level that falls within a range of accepted expression levels based on subject characteristics such as, e.g., a subject’s age, sex, weight, height, ethnicity, lifestyle, and/or one or more comorbidities. A sub-therapeutic level may refer to an expression level that is below a physiological value, such that the sub-therapeutic expression level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% lower than a physiological value. A sub-therapeutic level may refer to an expression level that is below a therapeutic level, such that the sub-therapeutic expression level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% lower than a therapeutic level.

As used herein, “administration” refers to dispensing, delivering, or applying a composition of the disclosure to a subject by any suitable route for delivery of the composition (e.g., a polynucleotide or a vector; e.g., an AAV comprising a transgene as described herein or a plurality thereof, an inhibitory RNA or a plurality thereof), to the desired location in the subject. Exemplary routes of administration include intramuscular, subcutaneous, intradermal, intravenous, intraperitoneal, intraarterial, intraventricular, intranasal, intraorbital, intracranial, or intraosseous administration.

As used herein, the term “administered in combination” or “combined administration” means that two or more agents are administered to a subject at the same time or within an interval such that there may be an overlap of an effect of each agent on the subject in need thereof. In some embodiments, the agents are administered within about 1 or more weeks, 1 or more days, 1 or more hours, or 1 or more minutes of one another. In some embodiments, the administrations of the agents are spaced sufficiently closely together such that a combinatorial (e.g., a synergistic) effect is achieved.

As used herein, the term “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable

for use in contact with the tissues a subject, such as a mammal (e.g., a human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, the term “pharmaceutically acceptable excipient,” as used herein, refers to any ingredient other than active agents (e.g., as described herein) present in pharmaceutical compositions and having the properties of being substantially nontoxic and non-inflammatory in subjects.

As used herein, the term “pharmaceutical composition” refers to a mixture containing a therapeutic agent, optionally in combination with one or more pharmaceutically acceptable excipients, diluents, and/or carriers, to be administered to a subject, such as a mammal, e.g., a human, in order to treat, reduce the likelihood of, or otherwise control a particular disease or condition affecting or that may affect the subject.

DETAILED DESCRIPTION

Described herein are compositions and methods for using heterologous gene expression technology. For example, the compositions and methods described herein are directed to expressing a polynucleotide comprising a transgene of interest in a host and then subsequently reducing the expression as needed via administering one or more inhibitory nucleic acid molecules.

Advantageously, the compositions and methods described herein permit temporal modulation of transgene expression. These compositions and methods of use thereof may be applied for tunable expression of a protein, such as a therapeutic protein, in the context of gene or cellular therapies.

I. Delivery of a Transgene for Heterologous Expression

Featured below are various compositions, such as nucleic acid molecules (e.g., transgenes for heterologous expression of a protein of interest or fragment thereof; e.g., a therapeutic protein or fragment thereof) and vectors or suitable compositions and methods for delivering said nucleic acid molecule to a host cell. All of these compositions and pharmaceutical compositions thereof and described methods are useful for methods of treatment described herein.

A. Transgene for Heterologous Expression

A transgene for heterologous gene expression may be delivered to a host cell (e.g., a host cell in a subject having or at risk of a disease or condition; e.g., a human subject) for heterologous expression of a polypeptide, a protein, or a fragment thereof (e.g., one or more protein domains, one or more protein chains, or a protein with one or more amino acid deletions). In some embodiments, a transgene for heterologous expression may encode a polypeptide, a protein, or a protein fragment of a soluble protein, a transmembrane protein, a membrane-associated protein, an intracellular protein, a secreted protein, or a fragment thereof.

A transgene for heterologous expression may be delivered to a host cell (e.g., a host cell in a subject having or at risk of a disease or condition; e.g., a human subject) as a method of therapy (e.g., a gene therapy). In some embodiments, the transgene is identified as a gene that underlies a

disease or a condition, such as a gene or a disease or condition set forth in Table 1. In some embodiments, the polypeptide, protein, or fragment thereof is an enzyme (e.g., a protease, a cellulase, a lipase, a hemicellulase, a laccase, an amylase, a glucoamylases, an esterase, a lactase, a polygalacturonase, a galactosidase, a ligninase, an oxidase, a peroxidase, an isomerase, a nitrilase, a hydroxylase, a polymerase, and a depolymerase), a growth factor, an immunomodulator, a cytokine, an antibody, a hormone, a transport protein, a contractile protein, an adhesion protein, a cell junction, or a surface receptor (e.g., an adhesion receptor, a G-protein coupled receptor, a channel, or a transporter). In some embodiments, a transgene for heterologous expression encodes a polypeptide, a protein, or a fragment thereof for ubiquitous or systemic expression (e.g., in all cells, organ systems, or tissues). In some embodiments, a transgene for heterologous expression is expressed in one or more organ systems or a subset of tissues or cell types therein.

In some embodiments, a transgene encodes a polypeptide, a protein, or a fragment thereof that shares 100% identity to the native protein sequence or a segment of equal length. In some embodiments, a transgene encodes a polypeptide, a protein, or a fragment thereof that shares 75% identity, 76% identity, 77% identity, 78% identity, 79% identity, 80% identity, 81% identity, 82% identity, 83% identity, 84% identity, 85% identity, 86% identity, 87% identity, 88% identity, 89% identity, 90% identity, 91% identity, 92% identity, 93% identity, 94% identity, 95% identity, 96% identity, 97% identity, 98% identity, 99% identity to the native protein sequence or a segment of equal length. In some embodiments, a transgene encodes a polypeptide, a protein, or a fragment thereof that is engineered or modified to have one or more mutations associated with enhanced expression, biodistribution, stability, and/or activity (e.g., enhanced binding affinity to one or more binding partners, enhanced catalysis or enzymatic activity). In some embodiments, a transgene for heterologous expression encodes a fusion protein such as, e.g., an Fc-fusion protein or an albumin-fusion protein, which may further enhance protein stability, protein biodistribution, and/or protein half-life.

A polynucleotide sequence of a transgene for heterologous expression may be modified to include a binding site or a region of complementarity for hybridization of one or more inhibitory RNA molecules. In preferred embodiments, the polynucleotide sequence of a transgene that includes a binding site or a region of complementarity for hybridization of one or more inhibitory RNA molecules is present in the transcribed mRNA transcript of the transgene. In some embodiments, a region of a transgene is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% complementary to one or more inhibitory RNA molecules. In some embodiments, a region of a transgene contains a polynucleotide sequence complementary to at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous within a region of an inhibitory RNA molecule.

In some embodiments, the binding site or region of complementarity for an inhibitory RNA molecule does not share sequence identity to an endogenous polynucleotide sequence of a host cell (e.g., a region of a host genome, a native nucleic acid; e.g., a native mRNA molecule). In some embodiments, the binding site or region of complementarity for an inhibitory molecule is in an intron or

a region therein of the transgene (e.g., the mRNA transcript of the transgene). In some embodiments, the binding site or region of complementarity for an inhibitory RNA molecule is in an exon or a region therein of the transgene (e.g., the mRNA transcript of the transgene). In some embodiments, the binding site or region of complementarity for an inhibitory molecule is in an exon or a region therein of the transgene (e.g., the mRNA transcript of the transgene). In some embodiments, the binding site or region of complementarity for an inhibitory RNA molecule is in an untranslated region (UTR) or a region therein of the transgene such as the 5' UTR or the 3' UTR of the transgene (e.g., the mRNA transcript of the transgene). In some embodiments, the binding site or region of complementarity for an inhibitory RNA molecule is about 10 bps, about 20 bps, about 30 bps, about 40 bps, about 50 bps, about 60 bps, about 70 bps, about 80 bps, about 90 bps, about 100 bps, about 110 bps, about 120 bps, about 130 bps, about 140 bps, or about 150 bps upstream of the start codon of the transgene (e.g., the mRNA transcript of the transgene). In some embodiments, the binding site or region of complementarity for an inhibitory RNA molecule is about 10 bps, about 20 bps, about 30 bps, about 40 bps, about 50 bps, about 60 bps, about 70 bps, about 80 bps, about 90 bps, about 100 bps, about 110 bps, about 120 bps, about 130 bps, about 140 bps, or about 150 bps downstream of the stop codon of the transgene (e.g., the mRNA transcript of the transgene).

B. Viral Genomes for Delivering a Transgene

Viral genomes provide a rich source of vectors that can be used for the efficient delivery of a transgene of interest into a host cell (e.g., a host cell in a subject having or at risk of a disease or condition; e.g., a human subject). Viral genomes are particularly useful vectors for gene delivery because the polynucleotides contained within such genomes are typically incorporated into the genome of a target 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 that may be used in conjunction with the compositions and methods described herein are adeno-associated virus (AAV), retrovirus, adenovirus (e.g., Ad5, Ad26, Ad34, Ad35, and Ad48), parvovirus (e.g., adeno-associated viruses), 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 that may be used in conjunction with the compositions and methods described herein include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include: avian leukosis-sarcoma, mammalian C-type, B-type viruses, D-type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., *Retroviridae: The viruses and their replication*, In *Fundamental Virology*, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996). Other examples include 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 US Patent No. 5,801,030, the disclosure of which is incorporated herein by reference as it pertains to viral vectors for use in gene therapy.

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C. AAV Vectors for Delivering a Transgene of Interest

In some embodiments, a transgene of interest is incorporated into recombinant AAV (rAAV) vectors in order to facilitate their introduction into a cell. rAAV vectors useful in the conjunction with the compositions and methods described herein include recombinant nucleic acid constructs that
10 contain (1) a polynucleotide such as transgene encoding a protein or fragment thereof and (2) one or more nucleic acids that facilitate expression of the polynucleotide. The viral nucleic acids may include those cis-acting elements of rAAV for replication and packaging (e.g., functional ITRs) of the DNA into a virion. Such rAAV vectors may also contain marker or reporter genes. Useful rAAV vectors include those having one or more of the naturally occurring AAV genes deleted in whole or in part but retain
15 functional flanking ITR sequences. The AAV ITRs may be of any serotype (e.g., derived from serotype 2) suitable for a particular application. Methods for using rAAV vectors are described, for example, in Tal et al. (*J. Biomed. Sci.* 7:279-291, 2000), and Monahan and Samulski (*Gene Delivery*. 7:24-30, 2000), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

20 The nucleic acids and vectors described herein can be incorporated into a rAAV virion in order to facilitate introduction of the nucleic acid or vector into a cell. The capsid proteins of AAV compose the exterior, non-nucleic acid portion of the virion and are encoded by the AAV cap gene. The cap gene encodes three viral coat proteins, VP1, VP2 and VP3, which are required for virion assembly. The construction of rAAV virions has been described, for example, in US Patent Nos.
25 5,173,414; 5,139,941; 5,863,541; 5,869,305; 6,057,152; and 6,376,237; as well as in Rabinowitz et al. (*J. Virol.* 76:791-801, 2002) and Bowles et al. (*J. Virol.* 77:423-432, 2003), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

rAAV virions useful in conjunction with the compositions and methods described herein include those derived from a variety of AAV serotypes including AAV1, AAV2, AAV3, AAV4, AAV5,
30 AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and AAV13, among others. Construction and use of AAV vectors and AAV proteins of different serotypes are described, for example, in Chao et al., *Mol. Ther.* 2:619-623, 2000), Davidson et al. (*Proc. Natl. Acad. Sci. USA* 97:3428-3432, 2000), Xiao et al. (*J. Virol.* 72: 2224-2232, 1998), Halbert et al. (*J. Virol.* 74: 1524-1532, 2000); Halbert et al. (*J. Virol.* 75: 6615-6624, 2001), and Auricchio et al. (*Hum. Molec. Genet.* 10: 3075-3081, 2001), the
35 disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

The nucleic acids and vectors described herein may also comprise a promoter sequence to initiate expression of a gene (e.g., a transgene). In some embodiments, the promoter is a constitutive promoter. In some embodiments, the promoter is an inducible promoter such as, e.g., a tetracycline-inducible promoter that induces gene expression in the presence of tetracycline or doxycycline. In
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some embodiments, the promoter is a naturally occurring promoter, such that the promoter is found in an organism (e.g., a target organism intended to receive delivery or administration of one or more polynucleotides described herein). In some embodiments, the promoter is naturally occurring such that it is native to a gene comprising the transgene of interest. In further embodiments, the promoter is a synthetic promoter. Additional exemplary promoters that are useful for the expression of a transgene include, but are not limited to, a respiratory syncytial virus (RSV) promoter, a cytomegalovirus (CMV) promoter, an elongation factor 1a (EF1a) promoter, a simian virus 40 (SV40) promoter, a muscle creatine kinase (MCK) promoter, a desmin promoter, a myosin light-chain (MLC) promoter, a cardiac troponin T (cTnT) promoter, a synapsin (Syn) promoter, a chicken β -actin promoter with CMV enhancer elements (CB7), a tetracycline-controlled transactivator protein (tTA) promoter, an upstream activating sequence (UAS) promoter, a homeobox protein 9 (HB9) promoter, a CD68 molecule (CD68) promoter, a platelet-derived growth factor beta chain promoter, a reverse tetracycline-controlled transactivator protein (rTA) promoter, a U1 promoter, a U6 promoter, and a U7 promoter, or variations thereof.

Also useful in conjunction with the compositions and methods described herein are pseudotyped rAAV vectors. Pseudotyped vectors include AAV vectors of a given serotype (e.g., AAV2) pseudotyped with a capsid gene derived from a serotype other than the given serotype (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and AAV13, among others). For example, a representative pseudotyped vector is an AAV2 vector encoding a therapeutic protein pseudotyped with a capsid gene derived from AAV serotype 8 or AAV serotype 9. In some embodiments, the pseudotyped AAV has the ITRs of one AAV serotype (e.g., AAV2) and the VP1, VP2, and/or VP3 capsid proteins from a different AAV serotype (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, or AAVrh74). Techniques involving the construction and use of pseudotyped rAAV virions are known in the art and are described, for example, in Duan et al., *J. Virol.* 75:7662-7671 (2001); Halbert et al. (*J. Virol.* 74:1524-1532, 2000); Zolotukhin et al. (*Methods.* 28:158-167, 2002); and Auricchio et al. (*Hum. Molec. Genet.* 10:3075-3081, 2001).

In some embodiments, the AAV comprises a capsid disclosed, e.g., in WO 2017/218842, the disclosure of which is incorporated herein by reference. In some embodiments, the AAV comprises a capsid protein disclosed in Lin et al. (*Mol Brain.* 13:138, 2020), the disclosure of which is incorporated herein by reference. In some embodiments, the AAV comprises an AAV2-retro or an AAV9-retro capsid protein. In some embodiments, the AAV comprises a capsid protein that is conjugated to a ligand or an aptamer.

AAV virions that have mutations within the virion capsid may be used to infect particular cell types more effectively than non-mutated capsid virions. For example, suitable AAV mutants may have ligand insertion mutations for the facilitation of targeting AAV to specific cell types. The construction and characterization of AAV capsid mutants including insertion mutants, alanine screening mutants, and epitope tag mutants is described in Wu et al. (*J. Virol.* 74:8635-45, 2000). Other rAAV virions that can be used in methods of the invention include those capsid hybrids that are generated by molecular breeding of viruses as well as by exon shuffling. See, e.g., Soong et al. (*Nat. Genet.* 25:436-439,

2000) and Kolman and Stemmer (*Nat. Biotechnol.* 19:423-428, 2001).

D. Additional Methods of Delivering a Transgene to a Host Cell

Techniques that can be used to introduce a transgene as described herein into a host cell (e.g., a host cell in a subject having or at risk of a disease or condition; e.g., a human subject) are known in the art. For example, electroporation can be used to permeabilize mammalian cells (e.g., human cells) by the application of an electrostatic potential to the cell of interest. Mammalian cells, such as human cells, subjected to an external electric field in this manner are subsequently predisposed to the uptake of exogenous nucleic acids. Electroporation of mammalian cells is described in detail, e.g., in Chu et al. (*Nucleic Acids Res.* 15: 1311, 1987), the disclosure of which is incorporated herein by reference. A similar technique, NUCLEOFECTION™, utilizes an applied electric field in order to stimulate the uptake of exogenous polynucleotides into the nucleus of a eukaryotic cell. NUCLEOFECTION™ and protocols useful for performing this technique are described in detail, e.g., in Distler et al. (*Exp. Dermatol.* 14:315, 2005), as well as in US 2010/0317114, the disclosures of each of which are incorporated herein by reference.

Additional techniques useful for the transfection of target cells include the squeeze-poration methodology. This technique induces the rapid mechanical deformation of cells in order to stimulate the uptake of exogenous DNA through membranous pores that form in response to the applied stress. This technology is advantageous in that a vector is not required for delivery of nucleic acids into a cell, such as a human target cell. Squeeze-poration is described in detail, e.g., in Sharei et al. (*J. Vis. Exp.* 81:e50980, 2013), the disclosure of which is incorporated herein by reference.

Lipofection represents another technique useful for transfection of target cells. This method involves the loading of nucleic acids into a liposome, which often presents cationic functional groups, such as quaternary or protonated amines, towards the liposome exterior. This promotes electrostatic interactions between the liposome and a cell due to the anionic nature of the cell membrane, which ultimately leads to uptake of the exogenous nucleic acids, for example, by direct fusion of the liposome with the cell membrane or by endocytosis of the complex. Lipofection is described in detail, for example, in US Patent No. 7,442,386, the disclosure of which is herein incorporated by reference. Similar techniques that exploit ionic interactions with the cell membrane to provoke the uptake of foreign nucleic acids include contacting a cell with a cationic polymer-nucleic acid complex. Exemplary cationic molecules that associate with polynucleotides so as to impart a positive charge favorable for interaction with the cell membrane are activated dendrimers (described, e.g., in Dennig (*Topics in Current Chemistry* 228:227, 2003), the disclosure of which is incorporated herein by reference) and diethylaminoethyl (DEAE)-dextran, the use of which as a transfection agent is described in detail, for example, in Gulick et al. (*Curr. Protoc. in Mol. Biol.* 40:1:9.2:9.2.1, 1997), the disclosure of which is incorporated herein by reference. Magnetic beads are another tool that can be used to transfect target cells in a mild and efficient manner, as this methodology utilizes an applied magnetic field in order to direct the uptake of nucleic acids. This technology is described in detail, for example, in US 2010/0227406, the disclosure of which is incorporated herein by reference.

Another useful tool for inducing the uptake of exogenous nucleic acids by target cells is

laserfection, a technique that involves exposing a cell to electromagnetic radiation of a particular wavelength in order to gently permeabilize the cells and allow polynucleotides to penetrate the cell membrane. This technique is described in detail, e.g., in Rhodes et al. (*Methods in Cell Biology* 82:309, 2007), the disclosure of which is incorporated herein by reference.

5 Microvesicles represent another potential vehicle that can be used to modify the genome of a target cell according to the methods described herein. For example, microvesicles that have been induced by the co-overexpression of the glycoprotein VSV-G with, e.g., a genome-modifying protein, such as a nuclease, can be used to efficiently deliver proteins into a cell that subsequently catalyze the site-specific cleavage of an endogenous polynucleotide sequence so as to prepare the genome of
10 the cell for the covalent incorporation of a polynucleotide of interest, such as a gene or regulatory sequence. The use of such vesicles, also referred to as Gesicles, for the genetic modification of eukaryotic cells is described in detail, e.g., in Quinn et al., *Mol. Ther.* 23: Supplement 1, Abstract No. 122, 2015).

15 **E. Methods of Genetic Engineering of a Host Cell**

In addition to the methods described herein, a host cell may be modified to comprise a transgene via a targeted integration method or other suitable gene editing technologies. Such methods may include the use of a site-specific nuclease, a transposase, a transcription activator-like effector nuclease (TALEN), meganuclease, zinc finger nuclease, a CRISPR/Cas9-based approach,
20 homologous recombination, prime editing, transposon mediated delivery, in which an exogenous polynucleotide (e.g., a polynucleotide comprising a transgene that encodes a protein of interest; e.g., a polynucleotide comprising a transgene and a region of complementarity to one or more inhibitory nucleic acids) may be integrated into the genome of a host cell.

25 **i. CRISPR**

A host cell may be modified to comprise a polynucleotide that comprises a transgene and/or a region of complementarity to one or more inhibitory nucleic acids using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology. "CRISPR" is programmable technology that targets specific stretches of genetic code to edit DNA at precise locations. CRISPR technology
30 may include CRISPR-Cas9. Cas9 (or "CRISPR-associated protein 9") is a nuclease that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence, allowing for the insertion of exogenous nucleic acids into a cell's genome. For example, CRISPR-based gene editing techniques can be used to introduce into a host cell genome, a transgene that encodes a protein of interest and a region that has
35 complementarity to one or more inhibitory nucleic acids.

Exemplary CRISPR systems include those that utilize a Cas9 nuclease. Cas9 nuclease, together with CRISPR sequences, form the basis of a technology known as CRISPR-Cas9 that can be used to edit genes within organisms. CRISPR technology may include Class 1 CRISPR systems including type I (cas3), type III (cas10), and type IV and 12 subtypes. CRISPR technology may
40 include Class 2 CRISPR systems including type II (cas9), type V (cas12), type VI (cas13), and 9

subtypes. In some embodiments, CRISPR technology may involve CRISPR-Cas design tools which are computer software platforms and bioinformatics tools used to facilitate the design of guide RNAs (gRNAs) for use with the CRISPR/Cas gene editing system. For example, CRISPR-Cas design tools may include: CRISPRon, CRISPROff, Invitrogen TrueDesign Genome Editor, Breaking-Cas, Cas-OFFinder, CASTING, CRISPy, CCTop, CHOPCHOP, CRISPOR, sgRNA Designer, Synthego Design Tool, and the like. CRISPR technology may also be used as a diagnostic tool. For example, CRISPR-based diagnostics may be coupled to enzymatic processes, such as SHERLOCK-based Profiling of *in vitro* Transcription (SPRINT). SPRINT can be used to detect a variety of substances, such as metabolites in subject samples or contaminants in environmental samples, with high throughput or with portable point-of-care devices.

Without being bound by theory, the mechanism of action of certain CRISPR nucleases includes the step of forming an R-loop whereby the CRISPR nuclease induces the unwinding of a double-strand DNA target, thereby separating the strands in the region bound by the CRISPR nuclease. The guide RNA spacer then hybridizes to the target strand at the protospacer sequence. This displaces a non-target strand that is complementary to the target strand, which forms the single strand region of the R-loop.

In some embodiments, the CRISPR nuclease includes one or more nuclease activities, which then cut the DNA leaving various types of lesions. For example, the CRISPR nuclease may comprise a nuclease activity that cuts the non-target strand at a first location, and/or cuts the target strand at a second location. In some embodiments, a CRISPR nuclease can cut zero, one, or two strands of a target nucleic acid. In some embodiments, the CRISPR nuclease is a nickase, which cuts one strand of a target nucleic acid. In some embodiments, the CRISPR nuclease is catalytically dead, which cuts zero strands of a target nucleic acid.

In some embodiments, the CRISPR nuclease comprises any one of the amino acid sequences as set forth herein. In some embodiments the CRISPR nuclease comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth herein.

Examples of CRISPR nucleases include, without limitation, Cas9 (e.g., catalytically dead Cas9 (dCas9) and nickase Cas9 (nCas9)), Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, and Cas12j/CasΦ, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas8a, Cas8b, Cas8c, Cas9 (also known as Csn1 or Csx12), Cas10, Cas10d, Csy1, Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csx11, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, homologs thereof, or modified or engineered versions thereof.

In some embodiments, the CRISPR system comprises a gene editor. In some embodiments, the gene editor comprises a CRISPR nuclease. The CRISPR nuclease of the gene editor may be any

CRISPR nuclease described herein. In some embodiments, the CRISPR nuclease of the gene editor is a nCas. In some embodiments, the CRISPR nuclease of the base editor is a dCas. In some embodiments, the gene editor of the disclosure further comprises an (nuclear localization signal) NLS domain.

5 In some embodiments, the gene editor is a fusion protein, and the components of the gene editor are domains of the fusion protein, optionally connected by a linker. In some embodiments, the gene editor is a multi-protein complex, and the components of the gene editor are provided as individual polypeptides. In some embodiments, the gene editor is a multi-protein complex, and one or more components of the gene editor are provided endogenously by the cell.

10 In some embodiments, the CRISPR system comprises a prime editor. In some embodiments, the prime editor comprises a CRISPR nuclease and a reverse transcriptase (RT) polypeptide. In some embodiments, the gRNA for use with the prime editor is a prime editing gRNA (PEgRNA). The CRISPR nuclease of the gene editor may be any CRISPR nuclease described herein. In some embodiments, the CRISPR nuclease of the gene editor is a nCas. In some embodiments, the
15 CRISPR nuclease of the base editor is a dCas. In some embodiments, the prime editors further comprise a flap endonuclease polypeptide. In some embodiments, the flap endonuclease polypeptide of the prime editor is a FEN1 domain. In some embodiments, the prime editors further comprise an NLS sequence.

In some embodiments, the prime editor is a fusion protein, and the components of the prime
20 editor are domains of the fusion protein, optionally connected by a linker. In some embodiments, the prime editor is a multi-protein complex, and the components of the prime editor are provided as individual polypeptides. In some embodiments, the prime editor is a multi-protein complex, and one or more components of the prime editor are provided endogenously by the cell.

25 **ii. Transposon System**

A host cell may be modified to comprise a polynucleotide that comprises a transgene and/or a region of complementarity to one or more inhibitory nucleic acids using a transposon system. Transposons or transposable elements include a (short) nucleic acid sequence with terminal repeat sequences upstream and downstream thereof. Transposons are polynucleotides that encode
30 transposase enzymes and contain a polynucleotide sequence (e.g., a polynucleotide sequence that comprises a transgene and a region of complementarity to one or more inhibitory nucleic acids) flanked by excision sites at the 5' and 3' positions. Once a transposon has been delivered into a cell, expression of the transposase gene commences and results in active enzymes that cleave the polynucleotide from the transposon. This activity is mediated by the site-specific recognition of
35 transposon excision sites by the transposase.

In some embodiments, these excision sites may be terminal repeats or inverted terminal repeats. Once excised from the transposon, the polynucleotide (e.g., that polynucleotide that comprises a transgene and a region that has complementarity to one or more inhibitory nucleic acids) can be integrated into the genome of a host cell by transposase-catalyzed cleavage of similar
40 excision sites that exist within nuclear genome of the cell. This allows the polynucleotide to be

inserted into the cleaved nuclear DNA at the excision sites, and subsequent ligation of the phosphodiester bonds that join the polynucleotide of interest to the DNA of the host cell genome completes the incorporation process. In some embodiments, the transposon may be a retrotransposon, such that the polynucleotide (e.g., a DNA sequence that comprises a transgene and a region that has complementarity to one or more inhibitory nucleic acids) is first transcribed to an RNA product and then reverse-transcribed to DNA before incorporation in the prokaryotic or eukaryotic cell genome. Exemplary transposon systems include the piggyBac transposon, the Sleeping Beauty transposon, the Frog Prince transposon, and the Tol2 transposon.

10 II. Inhibitory Nucleic Acids

Featured below are inhibitory nucleic acid molecules including small interfering RNAs, double-stranded RNAs, microRNAs, short hairpin RNAs, antisense oligonucleotides, and gapmers, as well as exemplary modifications thereof that may be used to reduce the expression of a transgene or an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) delivered by any one or more of the vectors (e.g., AAV vectors) described herein. All of these inhibitory nucleic acid molecules, modifications thereof, and pharmaceutical compositions thereof are useful for the methods, such as the methods of treatment, described herein.

20 A. Small interfering RNAs

A small interfering RNA (siRNA) molecule is a single-stranded (ss) or double-stranded (ds) nucleic acid molecule made of DNA, RNA, or both DNA and RNA (e.g., a chimeric) nucleosides that are complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest), or a region therein, and prevents translation of the mRNA into a protein. Once an siRNA molecule enters a cell, it is incorporated into an RNA-induced silencing complex (RISC). Upon hybridization of an antisense strand of the siRNA molecule to a region in an mRNA molecule following transcription of the transgene, the RISC complex will cleave the target mRNA, thereby inactivating the target mRNA, resulting in reduced mRNA and protein levels of the target. In some instances, an siRNA inhibits translation of the mRNA into a protein via translation repression or deadenylation-dependent decay mechanisms.

In some embodiments, an siRNA molecule may include a nucleotide sequence of about 10 to about 30 nucleotides in length (e.g., 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, or 31 nucleotides in length).

In some embodiments, an siRNA molecule of the disclosure may include a nucleotide sequence of 10 to 30 nucleotides in length (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length).

It is within the scope of the disclosure that any length, known and previously unknown in the art, may be employed for the current invention.

In some embodiments, an siRNA molecule contains an antisense strand. In some embodiments, the length of the antisense strand is between 10 and 30 nucleotides (e.g., 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), 15 and 25 nucleotides (e.g., 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides,), or 18 and 23 nucleotides (e.g., 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, or 23 nucleotides). In some embodiments, the antisense strand is 20 nucleotides. In some embodiments, the antisense strand is 21 nucleotides. In some embodiments, the antisense strand is 22 nucleotides. In some embodiments, the antisense strand is 23 nucleotides. In some embodiments, the antisense strand is 24 nucleotides. In some embodiments, the antisense strand is 25 nucleotides. In some embodiments, the antisense strand is 26 nucleotides. In some embodiments, the antisense strand is 27 nucleotides. In some embodiments, the antisense strand is 28 nucleotides. In some embodiments, the antisense strand is 29 nucleotides. In some embodiments, the antisense strand is 30 nucleotides.

In some embodiments, an siRNA molecule contains a sense strand. In some embodiments, the sense strand is between 10 and 30 nucleotides (e.g., 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), or 14 and 23 nucleotides (e.g., 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, or 23 nucleotides). In some embodiments, the sense strand is 15 nucleotides. In some embodiments, the sense strand is 16 nucleotides. In some embodiments, the sense strand is 17 nucleotides. In some embodiments, the sense strand is 18 nucleotides. In some embodiments, the sense strand is 19 nucleotides. In some embodiments, the sense strand is 20 nucleotides. In some embodiments, the sense strand is 21 nucleotides. In some embodiments, the sense strand is 22 nucleotides. In some embodiments, the sense strand is 23 nucleotides. In some embodiments, the sense strand is 24 nucleotides. In some embodiments, the sense strand is 25 nucleotides. In some embodiments, the sense strand is 26 nucleotides. In some embodiments, the sense strand is 27 nucleotides. In some embodiments, the sense strand is 28 nucleotides. In some embodiments, the sense strand is 29 nucleotides. In some embodiments, the sense strand is 30 nucleotides.

In some embodiments, the sense and antisense strands of an siRNA molecule are completely complementary to one another (e.g., 100% complementary). In some embodiments, the sense and antisense strands of an siRNA molecule of the disclosure are completely complementary to the extent that their lengths overlap with one another. Depending on the sequence of the sense and antisense strand, complementarity need not be complete or perfect, which means that the sense and antisense strand do not hybridize at every base pair due to mismatches. One or more (e.g., 1, 2, 3, 4, or 5)

mismatches may be present within the ds siRNA molecule without impacting the siRNA molecule's ability to reduce the expression of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and ultimately the expression of a protein.

5 In some embodiments, an siRNA molecule contains a sequence complementary to at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides to a segment of equal length within a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) with which the siRNA molecule can hybridize to the mRNA transcript or a region therein.

The nucleotide sequence of an siRNA molecule may contain sufficient complementarity to a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) such that the siRNA molecule can hybridize with a region unique to the transcript and not hybridize with a region native or endogenous to a host cell (e.g., a host genome; e.g., a subject's genome). In some embodiments, the siRNA can hybridize with one or more regions unique to the transcript. In some embodiments, the siRNA molecule is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% complementary an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) or a region (e.g., a segment of equal length). In some embodiments, the siRNA molecule is 100% complementary to an mRNA transcript (e.g., a transcript transcribed from the transgene of interest; e.g., a transcript encoding a protein of interest), or a region therein.

In some embodiments, the nucleotide sequence of an siRNA molecule may contain sufficient complementarity to an exon sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), or a region therein. In some embodiments, the nucleotide sequence of an siRNA molecule may contain sufficient complementarity to an intron sequence within a transcript that encodes a protein of interest, or a portion thereof. In some embodiments, an siRNA molecule may contain sufficient complementarity to a pre-mRNA transcript or an mRNA transcript that a protein of interest. In some embodiments, the nucleotide sequence of an siRNA molecule may contain sufficient complementarity to an untranslated region (UTR) of the mRNA transcript that encodes a protein of interest. In some embodiments, the UTR of the mRNA transcript is the 5' UTR. In some embodiments, the UTR of the mRNA transcript is the 3' UTR. In some embodiments, an siRNA molecule may contain sufficient complementarity to a region upstream of the start codon of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), such that the siRNA molecule is complementary to a region that is about 10 bps, about 20 bps, about 30 bps, about 40 bps, about 50 bps, about 60 bps, about 70 bps, about 80 bps, about 90 bps, about 100 bps, about 110 bps, about 120 bps, about 130 bps, about 140 bps, or about 150 bps upstream of the start codon of the transcript. In some embodiments, an siRNA

molecule may contain sufficient complementarity to a region downstream of the stop codon of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), such that the siRNA molecule is complementary to a region that is about 10 bps, about 20 bps, about 30 bps, about 40 bps, about 50 bps, about 60 bps, about 70 bps, about 80 bps, about 90 bps, about 100 bps, about 110 bps, about 120 bps, about 130 bps, about 140 bps, or about 150 bps downstream of the stop codon of the mRNA transcript.

In some embodiments, an siRNA molecule described herein may have 0-7 nucleotide 3' overhangs or 0-4 nucleotide 5' overhangs. In some embodiments, the siRNA molecule has a single uracil overhang at one or more 3' ends of the siRNA. In some embodiments, the siRNA molecule has a double uracil overhang at one or more 3' ends of the siRNA. In some embodiments, the siRNA molecule has a single thymine overhang at one or more 3' ends of the siRNA. In some embodiments, the siRNA molecule has a double thymine overhang at one or more 3' ends of the siRNA. In some embodiments, the siRNA molecule has a single cytosine and a single thymine (e.g., CT) overhang at one or more 3' ends of the siRNA.

For any of the methods described herein, different siRNA molecules (e.g., two or more, three or more, four or more, or five or more different siRNA molecules) can be combined for reducing the expression of a transgene of interest (e.g., a therapeutic transgene that encodes a protein of interest; e.g., a transgene incorporated into an AAV vector described herein). A combination of two or more siRNA molecules, such as two different siRNA molecules, three different siRNA molecules, four different siRNA molecules, five different siRNA molecules, or more, may have overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and may be used in a method of the disclosure to inhibit said transcript. Alternatively, two different siRNA molecules, three different siRNA molecules, four different siRNA molecules, five different siRNA molecules, or more, may not have any overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and are instead complementary to distinct regions to a transcript and may be used in a method of the disclosure to inhibit the mRNA transcript.

B. Double-stranded RNAs

A double-stranded RNA (dsRNA) molecule of the disclosure is a double-stranded nucleic acid molecule made of DNA, RNA, or both DNA and RNA (e.g., a chimeric) nucleosides that are complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and prevents translation of the mRNA into a protein or polypeptide. Typically, a dsRNA molecule is longer than an siRNA molecule and is processed within a cell to form an siRNA molecule. The antisense strand of the siRNA molecule is then incorporated into RISC, which, upon siRNA hybridization to a target mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), will cleave the target mRNA transcript, thereby inactivating the target mRNA transcript and resulting in reduced expression levels of the mRNA transcript and protein (e.g.,

the protein of interest). In some instances, a dsDNA may prevent translation of the mRNA into a protein via translation repression mechanisms.

In some embodiments, a dsRNA molecule of the disclosure may include a sense strand and an antisense strand, each containing a nucleotide sequence of about 25 to about 5,000 nucleotides in
5 length, or longer.

It is within the scope of the disclosure that any length, known and previously unknown in the art, may be employed for the current invention.

In some embodiments, a dsRNA molecule of the disclosure contains a sequence complementary to at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16,
10 at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides within a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) with which the dsRNA molecule can hybridize.

In some embodiments, the sense and antisense strands of a dsRNA molecule are completely complementary to one another. In some embodiments, the sense and antisense strands of a dsRNA molecule of the disclosure are completely complementary to the extent that their lengths overlap with one another. Depending on the sequence of the sense and antisense strand, complementarity need not be complete or perfect, which means that the sense and antisense strand
15 do not hybridize at every base pair due to mismatches. One or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) mismatches may be present within the dsRNA molecule without impacting the dsRNA molecule's ability to reduce the expression of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA
20 transcript that encodes a protein of interest) and ultimately the expression of a protein.

The nucleotide sequence of a dsRNA molecule of the disclosure may contain sufficient complementarity to a portion of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) such that the dsRNA molecule can hybridize to the mRNA transcript or a region therein. The nucleotide sequence
30 of a dsRNA molecule may contain sufficient complementarity to a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) such that the dsRNA molecule can hybridize with a region unique to the transcript and not hybridize with a region native or endogenous to a host cell (e.g., a host genome; e.g., a subject's genome).

In some embodiments, the dsRNA molecule is at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) or a portion thereof. In some
35 40 embodiments, the dsRNA molecule is 100% complementary to an mRNA transcript (e.g., an mRNA

transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), or a region therein.

In some embodiments, the nucleotide sequence of a dsRNA molecule may contain sufficient complementarity to an exon sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), or a region therein. In some embodiments, the nucleotide sequence of a dsRNA molecule may contain sufficient complementarity to an intron sequence within an mRNA transcript that encodes a protein of interest, or a portion thereof. In some embodiments, a dsRNA molecule may contain sufficient complementarity to a pre-mRNA transcript or an mRNA transcript that encodes a protein of interest. In some embodiments, the nucleotide sequence of a dsRNA molecule may contain sufficient complementarity to an untranslated region (UTR) of the mRNA transcript that encodes a protein of interest. In some embodiments, the UTR of the mRNA transcript is the 5' UTR. In some embodiments, the UTR of the mRNA transcript is the 3' UTR. In some embodiments, a dsRNA molecule may contain sufficient complementarity to a region upstream of the start codon of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), such that the siRNA molecule is complementary to a region that is about 10 bps, about 20 bps, about 30 bps, about 40 bps, about 50 bps, about 60 bps, about 70 bps, about 80 bps, about 90 bps, about 100 bps, about 110 bps, about 120 bps, about 130 bps, about 140 bps, or about 150 bps upstream of the start codon of the transcript. In some embodiments, a dsRNA molecule may contain sufficient complementarity to a region downstream of the stop codon of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), such that the dsRNA molecule is complementary to a region that is about 10 bps, about 20 bps, about 30 bps, about 40 bps, about 50 bps, about 60 bps, about 70 bps, about 80 bps, about 90 bps, about 100 bps, about 110 bps, about 120 bps, about 130 bps, about 140 bps, or about 150 bps downstream of the stop codon of the mRNA transcript.

For any of the methods described herein, different dsRNA molecules (e.g., two or more, three or more, four or more, or five or more different dsRNA molecules) can be combined for reducing the expression of a transgene of interest (e.g., a therapeutic transgene that encodes a protein of interest; e.g., a transgene incorporated into an AAV vector described herein). A combination of two or more dsRNA molecules, such as two different siRNA molecules, three different dsRNA molecules, four different dsRNA molecules, five different dsRNA molecules, or more, may have overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and may be used in a method of the disclosure to inhibit said transcript. Alternatively, two different siRNA molecules, three different siRNA molecules, four different dsRNA molecules, five different dsRNA molecules, or more, may not have any overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and are instead complementary to distinct regions to an mRNA transcript and may be used in a method of the disclosure to inhibit the mRNA transcript.

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C. MicroRNAs

A microRNA (miRNA) molecule of the disclosure is a short, ss nucleic acid molecules made of DNA, RNA, or both DNA and RNA (e.g., a chimeric) nucleosides that are complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) or a region therein and prevents translation of the mRNA into a protein or polypeptide. Once an miRNA molecule enters a cell, it is incorporated into RISC, which, upon miRNA hybridization to a target mRNA (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) will cleave the target mRNA, thereby inactivating the target mRNA and resulting in reduced expression levels of the mRNA transcript and protein (e.g., the protein of interest).

In some embodiments, an miRNA molecule of the disclosure may include a nucleotide sequence of about 6 to about 30 nucleotides in length (e.g., 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, or 31 nucleotides in length).

In some embodiments, an miRNA may include a nucleotide sequence of 6 to 30 nucleotides in length (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length).

It is within the scope of the disclosure that any length, known and previously unknown in the art, may be employed for the current invention.

In some embodiments, an miRNA molecule contains a sequence complementary to at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides within a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest and that encodes the protein of interest). The nucleotide sequence of the miRNA molecule may contain sufficient complementarity to a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) such that the miRNA molecule can hybridize with the mRNA transcript. The nucleotide sequence of an miRNA molecule may contain sufficient complementarity to a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) such that the miRNA molecule can hybridize with a region unique to the transcript and not hybridize with a region native or endogenous to a host cell (e.g., a host genome; e.g., a subject's genome). In some embodiments, the siRNA can hybridize with one or more regions unique to the transcript. In some embodiments, the siRNA molecule is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% complementary an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) or a region (e.g., a segment of equal length). In some embodiments, the siRNA molecule is 100% complementary to a transcript (e.g., a transcript

transcribed from the transgene of interest; e.g., a transcript encoding a protein of interest) or a region therein.

In some embodiments, the miRNA molecule is at least 70%, at least 75%, at least 80%, least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%,
5 at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) or a region therein. In some
embodiments, the miRNA molecule is 100% complementary to an mRNA transcript (e.g., an mRNA
transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein
10 of interest) or a region therein.

In some embodiments, the nucleotide sequence of an miRNA molecule may contain sufficient complementarity to an exon sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) or a region therein. In some embodiments, the nucleotide sequence of a miRNA molecule may
15 contain sufficient complementarity to an intron sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest), or a region therein. In some embodiments, a miRNA molecule of the disclosure may contain sufficient complementarity to a pre-mRNA transcript or an mRNA transcript within an mRNA
transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA
20 transcript that encodes a protein of interest) or a region therein.

For any of the methods described herein, different miRNA molecules (e.g., two or more, three or more, four or more, or five or more different miRNA molecules) can be combined for reducing the expression of a transgene of interest (e.g., a therapeutic transgene that encodes a protein of interest; e.g., a transgene incorporated into an AAV vector described herein). A combination of two or more
25 miRNA molecules, such as two different miRNA molecules, three different miRNA molecules, four different miRNA molecules, five different miRNA molecules, or more, may have overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and may be used in a method of the disclosure to inhibit said transcript. Alternatively, two different miRNA molecules, three different
30 miRNA molecules, four different miRNA molecules, five different miRNA molecules, or more, may not have any overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and are instead complementary to distinct regions to an mRNA transcript and may be used in a method of the disclosure to inhibit the mRNA transcript.

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D. Short Hairpin RNAs

A short hairpin RNA (shRNA) molecule of the disclosure is a ss or ds nucleic acid molecule made of DNA, RNA, or both DNA and RNA (e.g., a chimeric) nucleosides that are complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an
40 mRNA transcript encoding a protein of interest) or a region therein, and prevents translation of the

mRNA transcript into a protein or polypeptide. Once a shRNA molecule enters a cell, it is incorporated into a RISC, which, upon shRNA hybridization to a target mRNA (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) or a region therein, the RISC complex will cleave the target mRNA, thereby inactivating the target mRNA and resulting in reduced mRNA and protein levels of the target.

In some embodiments, an shRNA molecule of the disclosure may include a nucleotide sequence of about 60 to about 100 nucleotides in length (e.g., 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, or 110 nucleotides in length).

In some embodiments, an shRNA molecule of the disclosure may include a nucleotide sequence of 60 to 100 nucleotides in length (e.g., 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, 96, 97, 98, 99, or 100 nucleotides in length).

In some embodiments, an shRNA molecule of the disclosure may contain a variable hairpin loop structure and a stem sequence. In some embodiments, the stem sequence may be 10 to 50 nucleotides in length (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length). In some embodiments, the hairpin size is between 4 to 50 nucleotides in length (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length), although the loop size may be larger without significantly affecting silencing activity. An shRNA molecule may contain mismatches, for example G-U mismatches between two strands of the shRNA stem without decreasing potency or the ability to reduce expression levels of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest). In some embodiments, an shRNA molecule is designed to include one or several G-U pairings in the hairpin stem to stabilize hairpins during propagation, for example.

The nucleotide sequence of an shRNA molecule of the disclosure may contain sufficient complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) or a region therein such that the shRNA molecule can hybridize with the mRNA transcript. The nucleotide sequence of an shRNA molecule may contain sufficient complementarity to a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) such that the shRNA molecule can hybridize with a region unique to the transcript and not hybridize with a region native or endogenous to a host cell (e.g., a host genome; e.g., a subject's genome). In some embodiments, the shRNA molecule is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) or a region therein. In some embodiments, the shRNA molecule is 100% complementary to an mRNA transcript (e.g., an mRNA

transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) or a region therein.

In some embodiments, the nucleotide sequence of an shRNA molecule of the disclosure may contain sufficient complementarity to an exon sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) or a region therein. In some embodiments, the nucleotide sequence of an shRNA molecule may contain sufficient complementarity to an intron sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) or a region therein. In some embodiments, an shRNA molecule of the disclosure may contain sufficient complementarity to a pre-mRNA transcript or an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) or a region therein.

For any of the methods described herein, different shRNA molecules (e.g., two or more, three or more, four or more, or five or more different shRNA molecules) can be combined for reducing the expression of a transgene of interest (e.g., a therapeutic transgene that encodes a protein of interest; e.g., a transgene incorporated into an AAV vector described herein). A combination of two or more shRNA molecules, such as two different shRNA molecules, three different shRNA molecules, four different shRNA molecules, five different shRNA molecules, or more, may have overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and may be used in a method of the disclosure to inhibit said transcript. Alternatively, two different shRNA molecules, three different shRNA molecules, four different shRNA molecules, five different shRNA molecules, or more, may not have any overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and are instead complementary to distinct regions to a transcript and may be used in a method of the disclosure to inhibit the mRNA transcript.

E. Antisense Oligonucleotides

An antisense oligonucleotide (ASO) is a ss nucleic acid molecule containing DNA nucleosides that are complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest) or a region therein, and prevents translation of the mRNA into a protein or polypeptide. Upon hybridization of an ASO to a target mRNA (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript encoding a protein of interest), RNase H will degrade the mRNA by hydrolyzation, resulting in reduced mRNA and protein levels of the target.

In some embodiments, an ASO of the disclosure may include a nucleotide sequence of about 12 to about 50 nucleotides in length (e.g., 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about

37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, or 51 nucleotides in length).

In some embodiments, an ASO of the disclosure may include a nucleotide sequence of 12 to 50 nucleotides in length (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,
5 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length).

It is within the scope of the disclosure that any length, known and previously unknown in the art, may be employed for the current invention.

In some embodiments, an ASO of the disclosure contains a sequence complementary to at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least
10 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30 at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50 contiguous nucleotides to a segment of equal length within a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the
15 transgene of interest; e.g., an mRNA transcript encoding a protein of interest) with which the ASO can hybridize to the mRNA transcript or a region therein.

The nucleotide sequence of an ASO may contain sufficient complementarity to a region of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a
20 transcript that encodes a protein of interest) such that the ASO can hybridize with a region unique to the transcript and not to a region native to a host genome (e.g., a subject's genome). In some embodiments, the ASO can hybridize with one or more regions unique to the mRNA transcript. In some embodiments, the ASO is at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to
25 an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein. In some embodiments, the ASO is 100% complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein.

In some embodiments, the nucleotide sequence of an ASO of the disclosure may contain
30 sufficient complementarity to an exon sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein. In some embodiments, the nucleotide sequence of an ASO may contain sufficient complementarity to an intron sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a
35 region therein. In some embodiments, an ASO of the disclosure may contain sufficient complementarity to a pre-mRNA transcript or an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein.

For any of the methods described herein, different ASOs (e.g., two or more, three or more,
40 four or more, or five or more different ASOs) can be combined for reducing the expression of a

transgene of interest (e.g., a therapeutic transgene that encodes a protein of interest; e.g., a transgene incorporated into an AAV vector described herein). A combination of two or more ASOs, such as two different ASOs, three different ASOs, four different ASOs, five different ASOs, or more, may have overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and may be used in a method of the disclosure to inhibit said transcript. Alternatively, two different ASOs, three different ASOs, four different ASOs, five different ASOs, or more, may not have any overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and are instead complementary to distinct regions to a transcript and may be used in a method of the disclosure to inhibit the mRNA transcript.

F. Gapmers

A gapmer is a single-stranded nucleic acid molecule containing an internal DNA region (i.e., a gap segment) flanked by one or two external RNA regions (i.e., wing segments). At a minimum, the gap segment contains a sequence complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein. Typically, the wing segments contain modified RNA; modified RNA (and DNA) is described further below. Upon the hybridization of a gapmer to a target mRNA (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein, RNase H will degrade the mRNA transcript by hydrolyzation, resulting in reduced mRNA and protein levels of the target.

In some embodiments, a gapmer of the disclosure may include a nucleotide sequence of about 10 to about 25 nucleotides in length (e.g., 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, or 26 nucleotides in length).

In some embodiments, a gapmer may include a nucleotide sequence of 10 to 25 nucleotides in length (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length).

It is within the scope of the disclosure that any length, known and previously unknown in the art, may be employed for the current invention.

In some embodiments, a gapmer of the disclosure contains at least one (e.g., 1 or 2) wing region. In some embodiments, the gapmer contains one wing region located 5' to the gap region. In some embodiments, the gapmer contains one wing region located 3' to the gap region. In some embodiments, the gapmer contains two wing regions, one located 5' to the gap region and the other located 3' to the gap region.

In some embodiments, a gapmer contains at least one (e.g., 1 or 2) wing region that is about 1 to about 7 nucleotides in length (e.g., about 1-8, about 1-7, about 1-6, about 1-5, about 1-4, about 1-3, or about 1-2 nucleotides in length). In some embodiments, the gapmer contains at least one (e.g., 1 or 2) wing region that is 1 nucleotide in length. In some embodiments, the gapmer contains at least one (e.g., 1 or 2) wing region that is 2 nucleotides in length. In some embodiments, the gapmer

contains at least one (e.g., 1 or 2) wing region that is 3 nucleotides in length. In some embodiments, the gapmer contains at least one (e.g., 1 or 2) wing region that is 4 nucleotides in length. In some embodiments, the gapmer contains at least one (e.g., 1 or 2) wing region that is 5 nucleotides in length. In some embodiments, the gapmer contains at least one (e.g., 1 or 2) wing region that is 6
5 nucleotides in length. In some embodiments, the gapmer contains at least one (e.g., 1 or 2) wing region that is 7 nucleotides in length. In some embodiments, the gapmer contains at least one (e.g., 1 or 2) wing region that is 8 nucleotides in length.

In some embodiments, a gapmer of the disclosure contains one gap region. In some embodiments, the gapmer contains one gap region located 5' to the wing region. In some
10 embodiments, the gapmer contains one gap region located 3' to the wing region. In some embodiments, the gapmer contains one gap region flanked by two wing regions.

In some embodiments, a gapmer of the disclosure contains at least one gap region that is about 8 to about 24 nucleotides in length (e.g., about 8-24, about 8-23, about 8-22, about 8-21, about 8-20, about 8-19, about 8-18, about 8-17, about 8-16, about 8-15, about 8-14, about 8-13, about 8-12,
15 about 8-10, or about 8-9 nucleotides in length).

The nucleotide sequence of the gapmer may contain sufficient complementarity to a portion of an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein such that the gapmer can hybridize with the mRNA transcript or a region therein. In some embodiments, the gapmer is at least 70%, at
20 least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein. In some embodiments, the gapmer is 100% complementary to an mRNA transcript
25 (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein.

In some embodiments, the nucleotide sequence of a gapmer may contain sufficient complementarity to an exon sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a
30 region therein. In some embodiments, the nucleotide sequence of a gapmer may contain sufficient complementarity to an intron sequence within an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein. In some embodiments, the nucleotide sequence of a gapmer of the disclosure may contain sufficient complementarity to a pre-mRNA transcript or an mRNA transcript (e.g., an mRNA
35 transcript transcribed from the transgene of interest; e.g., a transcript that encodes a protein of interest) or a region therein.

For any of the methods described herein, different gapmers (e.g., two or more, three or more, four or more, or five or more different gapmers) can be combined for reducing the expression of a transgene of interest (e.g., a therapeutic transgene that encodes a protein of interest; e.g., a
40 transgene incorporated into an AAV vector described herein). A combination of two or more gapmers,

such as two different gapmers, three different gapmers, four different gapmers, five different gapmers, or more, may have overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and may be used in a method of the disclosure to inhibit said transcript. Alternatively, two
5 different gapmers, three different gapmers, four different gapmers, five different gapmers, or more, may not have any overlapping regions of complementarity to an mRNA transcript (e.g., an mRNA transcript transcribed from the transgene of interest; e.g., an mRNA transcript that encodes a protein of interest) and are instead complementary to distinct regions to a transcript and may be used in a method of the disclosure to inhibit the mRNA transcript.

10

G. Modifications to Nucleic Acid Molecules

It is contemplated that any of the nucleic acid molecules described herein (e.g., inhibitory nucleic acid molecules) may be used in the methods disclosed herein in an unmodified or in a modified form. Unmodified nucleic acid molecules contain nucleobases that include the purine bases
15 adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleic acid molecules are described in more detail below.

Modifications may be achieved by systematically adding or removing linked nucleosides to generate longer or shorter sequences.

Modifications may be achieved by incorporating, for example, one or more alternative
20 nucleosides, alternative 2' sugar moieties, and/or alternative internucleoside linkages. Typically, these types of modifications are introduced to optimize the molecule's efficacy or biophysical properties (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, reduce immunogenicity, and/or targeting to a particular location or cell type). By way of example, a modified nucleotide such as a locked nucleic acid (LNA), a peptide nucleic acid
25 (PNA), or a bridged nucleic acid (BNA) may be incorporated into any nucleic acid-based inhibitor described above. Further nucleic acid modifications are described below.

Modification may further be achieved by covalently or non-covalently conjugating a moiety (e.g., a targeting moiety, a hydrophobic moiety, a cell penetrating peptide, or a polymer) to the 5' end and/or 3' end of the inhibitory nucleic acid molecule, as described in more detail below.

30

i. Nucleoside Modifications

Modification of the inhibitory nucleic acid molecules described herein include one or more of the following nucleoside modifications: 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and
35 guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH₃) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines,
40 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-

azaadenine, 7-deazaguanine and 7-deazaadenine, and/or 3-deazaguanine and 3-deazaadenine. The inhibitory nucleic acid molecules may also include nucleobases in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine, and/or 2-pyridone. Further modification of the inhibitory nucleic acid molecules

- 5 described herein may include nucleobases disclosed in US 3,687,808; Kroschwitz, J.I., ed. The Concise Encyclopedia of Polymer Science and Engineering, New York, John Wiley & Sons, 1990, pp. 858-859; Englisch et al., Angewandte Chemie, International Edition 30:613, 1991; and Sanghvi, Y.S., Chapter 16, Antisense Research and Applications, CRC Press, Gait, M.J. ed., 1993, pp. 289-302.

10 **ii. Sugar Modifications**

- Modifications of the inhibitory nucleic acid molecules described herein may also include one or more of the following 2' sugar modifications: 2'-O-methyl (2'-O-Me), 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE), 2'-dimethylaminoethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, and/or 2'-dimethylaminoethoxyethoxy (also
- 15 known in the art as 2'-O-dimethylamino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O-CH₂OCH₂N(CH₃)₂. Other possible 2'-modifications that can modify the inhibitory nucleic acid molecules described herein include all possible orientations of OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. Other potential sugar substituent groups include, e.g.,
- 20 aminopropoxy (-OCH₂CH₂CH₂NH₂), allyl (-CH₂-CH=CH₂), -O-allyl (-O-CH₂-CH=CH₂) and fluoro (F). 2'-sugar substituent groups may be in the arabino (up) position or ribo (down) position. In some embodiments, the 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the interfering RNA molecule, particularly the 3' position of the sugar on the 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide.
- 25 Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

iii. Internucleoside Linkage Modifications

- Modifications of the inhibitory nucleic acid molecules described herein may include one or
- 30 more of the following internucleoside modifications: phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates, and boranophosphates
- 35 having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage.

iv. Conjugates

- Any of the inhibitory nucleic acid molecules described herein may be modified via the addition
- 40 of an auxiliary moiety, e.g., a cell penetrating peptide (CPP), a polymer, a hydrophobic moiety, or a

targeting moiety. The auxiliary moiety may be present as a 5' terminal modification (e.g., covalently bonded to a 5'-terminal nucleoside), a 3' terminal modification (e.g., covalently bonded to a 3'-terminal nucleoside), or an internucleoside linkage (e.g., covalently bonded to phosphate or phosphorothioate in an internucleoside linkage).

- 5 CPPs are known in the art (e.g., TAT or Arg8) (Snyder and Dowdy, 2005, Expert Opin. Drug Deliv. 2, 43-51). Specific examples of CPPs are provided in WO2011157713, which is incorporated herein by reference in its entirety.

- Inhibitory nucleic acid molecules of the disclosure may include covalently attached neutral polymer-based auxiliary moieties. Neutral polymers include poly(C1-6 alkylene oxide), e.g.,
10 poly(ethylene glycol) and poly(propylene glycol) and copolymers thereof, e.g., di- and triblock copolymers.

- An inhibitory nucleic acid molecule containing a hydrophobic moiety may exhibit superior cellular uptake and/or pharmacokinetic properties, as compared to an inhibitory nucleic acid molecule lacking the hydrophobic moiety. A hydrophobic moiety is a monovalent group (e.g., a bile acid; e.g.,
15 cholic acid, taurocholic acid, deoxycholic acid, oleyl lithocholic acid, or oleyl cholenic acid), glycolipid, phospholipid, sphingolipid, isoprenoid, vitamin, saturated fatty acid, unsaturated fatty acid, fatty acid ester, triglyceride, cholesterol, pyrene, porphyrine, texaphyrine, adamantane, acridine, biotin, coumarin, fluorescein, rhodamine, Texas-Red, digoxigenin, dimethoxytrityl, t-butyldimethylsilyl, t-butyldiphenylsilyl, cyanine dye (e.g., Cy3 or Cy5), Hoechst 33258 dye, psoralen, or ibuprofen)
20 covalently linked to the nucleic acid backbone (e.g., 5'-terminus) of the inhibitory nucleic acid molecule. In some embodiments, a hydrophobic moiety is a cholesterol.

III. Pharmaceutical Compositions

- The nucleic acids and vectors described herein may be formulated into various compositions
25 (e.g., a pharmaceutical composition) for administration to a subject in a biologically compatible form suitable for administration *in vivo*. For example, the agents described herein may be administered in a suitable diluent, carrier, stabilizer, or excipient, and may further contain a preservative, e.g., to prevent the growth of microorganisms. Conventional procedures and ingredients for the selection and preparation of suitable compositions are described, for example, in Remington, J.P. The Science and
30 Practice of Pharmacy, Easton, PA. Mack Publishers, 2012, 22nd ed. and in The United States Pharmacopeial Convention, The National Formulary, United States Pharmacopeial, 2015, USP 38 NF 33.

- Mixtures of agents described herein may be prepared in water suitably mixed with one or more excipients, carriers, or diluents. Dispersions may also be prepared in glycerol, liquid
35 polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (described in US 5,466,468, the disclosure of which is incorporated herein by reference). In any case
40 the formulation may be sterile and may be fluid to the extent that easy syringability exists.

Formulations may be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils.

- 5 Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.
- 10 Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For example, a solution containing a pharmaceutical composition described herein may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intramuscular, intravenous,

15 subcutaneous, and intraperitoneal administration.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, e.g., to non-human animals, e.g., non-human mammals. Modification of

20 pharmaceutical compositions suitable for administration to humans to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates and mammals.

25 Compositions containing an agent such as a nucleic acid or an inhibitory nucleic acid molecule described herein may include a delivery vehicle for administration to a subject or one or more cells thereof. Exemplary delivery vehicles for an inhibitory nucleic acid molecule described herein include, but are not limited to, lipid-based carriers, lipid nanoformulations such as lipid nanoparticles (LNP), and suitable polymers.

30 Compositions containing an agent such as a nucleic acid or vector described herein may further include a second agent (e.g., a nucleic acid molecule to be expressed within a cell, a polypeptide, or a drug). For example, a second agent may be a blood pressure medication, steroid, an analgesic, or an immunosuppressive agent.

35 **IV. Methods of Treatment**

Any of the compositions such as the nucleic acid molecules (e.g., polynucleotides comprising transgenes, inhibitory nucleic acid molecules, and inhibitory RNA molecules), vectors and AAV vectors (e.g., rAAV vectors), and any pharmaceutical compositions thereof, can be used in a method of treatment for a disease or condition in a subject in need thereof (e.g., a human subject). In some

40 embodiments, a method of treatment may be prophylactic treatment for a subject at risk of a disease

or condition. In other embodiments, a method of treatment may reduce, reverse, ameliorate, stabilize, or improve a disease state or condition in a subject. In other embodiments, the methods may be used to alleviate, ameliorate, reduce, or reverse one or more clinical manifestations of a disease or condition.

- 5 In some embodiments, the method of treatment is directed to treating a metabolic disorder, a blood disorder, a cardiovascular disorder, a neurological disorder, an ocular or ophthalmological disorder, a reproductive disorder, an infectious disease, an autoimmune or immunological disorder, or a type of cancer. Exemplary diseases and target genes (e.g., suitable transgenes) that may benefit from the compositions and methods described herein are summarized in Table 1 below.

10 **Table 1: Exemplary Diseases and Target Genes**

Disease(s)	Gene(s)
Achondroplasia	FGFR3
Achromatopsia	CNGA3, CNGB3, GNAT2, PDE6C, PDE6H, ACHM2, ACHM3
Acute Renal Injury	NFkappaB, AATF, p85alpha, FAS, Apoptosis cascade elements (e.g., FASR, Caspase 2, 3, 4, 6, 7, 8, 9, 10, AKT, TNF alpha, IGF1, IGF1R, RIPK1), p53
Age Related Macular Degeneration	Abcr, CCL2, CC2, CP, Timp3, cathepsinD, VLDLR, CCR2, sFLT01 (VEGF/PIGF (placental growth factor) binding domain of human VEGFR1/Flt-1 (hVEGFR1) fused to the Fe portion of human IgG(I) through a polyglycine linker)
Acquired Immune Deficiency Syndrome (AIDS)	KIR3DL1, NKAT3, NKB1, AMB11, KIR3DS1, IFNG, CXCL12, SDF1
Albinism (including oculocutaneous albinism (Types 1-7) and ocular albinism)	TYR, OCA2, TYRP1, SLC45A2, SLC24A5, C10orf11
Alkaptonuria	HGD
Alpha-1 Antitrypsin Deficiency (AATD or AIAD) or Hereditary Emphysema	AAT, SERPINA1, those set forth in WO2017165862, PiZ allele

Disease(s)	Gene(s)
Amyotrophic lateral sclerosis (ALS)	SOD1, ALS2, ALS3, ALS5, ALS7, STEX, FUS, TARDBP, VEGF (VEGF-a; VEGF-b; VEGF-c), DPP6, NEFH, PTGS1, SLC1A2, TNFRSF10B, PRPH, HSP90AA1, CRIA2, IFNG, AMPA2 S100B, FGF2, AOX1, CS, TXN, RAPHJ1, MAP3K5, NBEAL1, GPX1, ICA1L, RAC1, MAPT, ITPR2, ALS2CR4, GLS, ALS2CR8, CNTFR, ALS2CR11, FOLH1, FAM117B, P4HB, CNTF, SQSTM1, STRADB, NAIP, NLR, YWHAQ, SLC33A1, TRAK2, SCA1, NIF3L1, NIF3, PARD3B, COX8A, CDK15, HECW1, HECT, C2, WW 15, NOS1, MET, SOD2, HSPB1, NEFL, CTSE, ANG, HSPA8, RNase A, VAPB, VAMP, SNCA, alpha HGF, CAT, ACTB, NEFM, TH, BCL2, FAS, CASP3, CLU, SMN1, G6PD, BAX, HSF1, RNF19A, JUN, ALS2CR12, HSPA5, MAPK14, APEX1, TXNRD1, NOS2, TIMP1, CASP9, XIAP, GLG1, EPO, VEGFA, ELN, GDNF, NFE2L2, SLC6A3, HSPA4, APOE, PSMB8, DCTN2, TIMP3, KIFAP3, SLC1A1, SMN2, CCNC, STUB1, ALS2, PRDX6, SYP, CABIN1, CASP1, GART, CDK5, ATXN3, RTN4, C1QB, VEGFC, HTT, PARK7, XDH, GFAP, MAP2, CYCS, FCGR3B, CCS, UBL5, MMP9m, SLC18A3, TRPM7, HSPB2, AKT1, DEERL1, CCL2, NGRN, GSR, TPPP3, APAF1, BTBD10, GLUD1, CXCR4, SLC1A3, FLT1, PON1, AR, LIF, ERBB3, GAS1, CD44, TP53, TLR3, GRIA1, GAPDH, AMPA, GRIK1, DES, CHAT, FLT4, CHMP2B, BAG1, CHRNA4, GSS, BAK1, KDR, GSTP1, OGG1, IL6
Alzheimer's Disease	E1, CHIP, UCH, UBB, Tau, LRP, PICALM, CLU, PS1, SORL1, CR1, VLDLR, UBA1, UBA3, CHIP28, AQP1, UCHL1, UCHL3, APP, AAA, CVAP, AD1, APOE, AD2, DCP1, ACE1, MPO, PACIP1, PAXIP1L, PTIP, A2M, BDNF, BLMH, BMH, PSEN1, AD3, ALAS2, ABCA1, BIN1, BDNF, BTNL8, C1ORF49, CDH4, CHRN2, CKLFSF2, CLEC4E, CR1L, CSF3R, CST3, CYP2C, DAPK1, ESR1, FCAR, FCGR3B, FFA2, FGA, GAB2, GALP, GAPDHS, GDNF, GMPB, HP, HTR7, IDE, IF127, IFI6, IFIT2, IL1RN, IL-1RA, IL8RA, IL8RB, JAG1, KCNJ15, LRP6, MAPT, MARK4, MPHOSPH1, MTHFR, NBN, NCSTN, NIACR2, NGF, NMNAT3, NTM, ORM1, P2RY13, PBEF1, PCK1, PICALM, PLA2, PLXNC1, PRNP, PSEN1, PSEN2, PTPRA, RALGPS2, RGS2, SELENBP1, SLC25A37, SORL1, Mitoferrin-1, TF, TFAM, TNF, TNFRSF10C, UBE1C
Amyloidosis	APOA1, APP, AAA, CVAP, AD1, GSN, FGA, LYZ, TTR, PALB
Amyloid Neuropathy	TTR, PALB
Anemia	CDAN1, CDA1, RPS19, DBA, PKLR, PK1, NT5C3, UMPH1, PSN1, RHAG, RH50A, NRAMP2, SPTB, ALAS2, ANH1, ASB, ABCB7, ABC7, ASAT
Angelman Syndrome	UBE3A
Anxiety	BDNF
Arthritis, Rheumatoid Arthritis	TNFR:Fc

Disease(s)	Gene(s)
Attention Deficit Hyperactivity Disorder (ADHD)	PTCHD1
Autoimmune Lymphoproliferative Syndrome	TNFRSF6, APT1, FAS, CD95, ALPS1A
Autism, Autism spectrum disorders (ASDs), including Asperger's and a general diagnostic category called Pervasive Developmental Disorders (PDDs)	PTCHD1, Mecp2, BZRAP1, MDGA2, Sema5A, Neurexin 1, GLO1, RTT, PPMX, MRX16, RX79, NLGN3, NLGN4, KIAA1260, AUTSX2, FMR1, FMR2, FXR1, FXR2, MGLUR5, ATP10C, CDH10, GRM6, MGLUR6, CDH9, CNTN4, NLGN2, CNTNAP2, SEMA5A, DHCR7, NLGN4X, NLGN4Y, DPP6, NLGN5, EN2, NRCAM, MDGA2, NRXN1, FMR2, AFF2, FOXP2, OR4M2, OXTR, FXR1, FXR2, PAH, GABRA1, PTEN, GABRA5, PTPRZ1, GABRB3, GABRG1, HIRIP3, SEZ6L2, HOXA1, SHANK3, IL6, SHBZRAP1, LAMB1, SLC6A4, SERT, MAPK3, TAS2R1, MAZ, TSC1, MDGA2, TSC2, MECP2, UBE3A, WNT2, see also 20110023145
Autosomal Dominant Polycystic Kidney Disease (ADPKD) - (includes diseases such as von Rippel-Lindau disease and Tuberous Sclerosis Complex disease)	PKD1, PKD2
Autosomal Recessive Polycystic Kidney Disease (ARPKD)	PKDH1
Ataxia-Telangiectasia (aka Louis Bar syndrome)	ATM
B-Cell Non-Hodgkin Lymphoma	BCL7A, BCL7
Bardet-Biedl Syndrome	ARL6, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, BBS10, BBS12, CEP290, INPP5E, LZTFL1, MKKS, MKS1, SDCCAGS, TRIM32, TTC8
Bare Lymphocyte Syndrome	TAPBP, TPSN, TAP2, ABCB3, PSF2, RING11, MHC2TA, C2TA, RFX5, RFXAP, RFX5
Bartter's Syndrome (Types I, II, III, IVA and B, and V)	SLC12A1 (type I), KCNJ1 (type II), CLCNKB (type III), BSND (type IV A), or both the CLCNKA CLCNKB genes (type IV B), CASR (type V)
Batten's Disease, Late Infantile Neuronal Ceroid Lipofuscinosis	CLN2
Becker Muscular Dystrophy	DMD, BMD, MYF6
Best Disease (Vitelliform Macular Dystrophy Type 2)	VMD2
Bipolar Disorder	BDNF
Bleeding Disorders	TBXA2R, P2RX1, P2XI
Blue Cone Monochromacy	OPN1LW, OPN1MW, LCR
Breast Cancer	BRCA1, BRCA2, COX-2
Bruton's Disease (aka X-linked Agammaglobulinemia)	BTk
Canavan's disease	ASPA
Cancers (e.g., Lymphoma, Chronic Lymphocytic Leukemia (CLL), B Cell Acute Lymphocytic Leukemia (B-ALL), Acute Lymphoblastic Leukemia, Acute Myeloid Leukemia, Non-Hodgkin's Lymphoma (NHL), Diffuse Large Cell Lymphoma (DLCL), Multiple Myeloma, Renal Cell Carcinoma (RCC), Neuroblastoma, Colorectal Cancer, Breast Cancer, Ovarian Cancer, Melanoma, Sarcoma, Lung Cancer, Esophageal Cancer, Hepatocellular Carcinoma, Pancreatic Cancer, Astrocytoma, Mesothelioma, Head and Neck Cancer, and Medulloblastoma)	FAS, BID, CTLA4, PDCD1, CBLB, PTPN6, TRAC, TRBC, those described in WO2015048577
Cardiovascular Diseases	IL1B, XDH, TP53, PTGS, MB, IL4, ANGPT1, ABCGu8, CTSK, PTGIR, KCNJ11, INS, CRP, PDGFRB, CCNA2, PDGFB, KCNJ5, KCNN3, CAPN10, ADRA2B, ABCG5, PRDX2, CPAN5,

Disease(s)	Gene(s)
	PARP14, MEX3C, ACE, RNF, IL6, TNF, STN, SERPINE1, ALB, ADIPOQ, APOB, APOE, LEP, MTHFR, APOA1, EDN1, NPPB, NOS3, PPARG, PLAT, PTGS2, CETP, AGTR1, HMGCR, IGF1, SELE, REN, PPARA, PON1, KNG1, CCL2, LPL, VWF, F2, ICAM1, TGFB, NPPA, IL10, EPO, SOD1, VCAM1, IFNG, LPA, MPO, ESR1, MAPK, HP, F3, CST3, COG2, MMP9, SERPINC1, F8, HMOX1, APOC3, IL8, PROL1, CBS, NOS2, TLR4, SELP, ABCA1, AGT, LDLR, GPT, VEGFA, NR3C2, IL18, NOS1, NR3C1, FGB, HGF, IL1A, AKT1, LIPC, HSPD1, MAPK14, SPP1, ITGB3, CAT, UTS2, THBD, F10, CP, TNFRSF11B, EGFR, MMP2, PLG, NPY, RHOD, MAPK8, MYC, FN1, CMA1, PLA2, GNB3, ADRB2, SOD2, F5, VDR, ALOX5, HLA- DRB1, PARP1, CD40LG, PON2, AGER, IRS1, PTGS1, ECE1, F7, IRMN, EPHX2, IGFBP1, MAPK10, FAS, ABCB1, JUN, IGFBP3, CD14, PDE5A, AGTR2, CD40, LCAT, CCR5, MMP1, TIMP1, ADM, DYT10, STAT3, MMP3, ELN, USF1, CFH, HSPA4, MMP12, MME, F2R, SELL, CTSB, ANXA5, ADRB1, CYBA, FGA, GGT1, LIPG, HIF1A, CXCR4, PROC, SCARB1, CD79A, PLTP, ADD1, FGG, SAA1, KCNH2, DPP4, NPR1, VTN, KIAA0101, FOS, TLR2, PIIG, IL1R1, AR, CYP1A1, SERPINA1, MTR, RBP4, APOA4, CDKN2A, FGF2, EDNRB, ITGA2, VLA-2, CABIN1, SHBG, HMGB1, HSP90B2P, CYP3A4, GJA1, CAV1, ESR2, LTA, GDF15, BDNF, CYP2D6, NGF, SP1, TGIF1, SRC, EGF, PIK3CG, HLA-A, KCNQ1, CNR1, FBN1, CHKA, BEST1, CTNNA1, IL2, CD36, PRKAB1, TPO, ALDH7A1, CX3CR1, TH, F9, CH1, TF, HFE, IL17A, PTEN, GSTM1, DMD, GATA4, F13A1, TTR, FABP4, PON3, APOC1, INSR, TNFRSF1B, HTR2A, CSF3, CYP2C9, TXN, CYP11B2, PTH, CSF2, KDR, PLA2G2A, THBS1, GCG, RHOA, ALDH2, TCF7L2, NFE2L2, NOTCH1, UGT1A1, IFNA1, PPARG, SIRT1, GNHR1, PAPP, ARR3, NPPC, AHSP, PTK2, IL13, MTOR, ITGB2, GSTT1, IL6ST, CPB2, CYP1A2, HNF4A, SLC64A, PLA2G6, TNFSF11, SLC8A1, F2RL1, AKR1A1, ALDH9A1, BGLAP, MTTP, MTRR, SULT1A3, RAGE, C4B, P2RY12, RNLS, CREB1, POMC, RAC1, LMNA, CD59, SCM5A, CYP1B1, MIF, MMP13, TIMP2, CYP19A1, CYP21A2, PTPN22, MYH14, MBL2, SELPLG, AOC3, CTSL1, PCNA, IGF2, ITGB1, CAST, CXCL12, IGHE, KCNE1, TFRC, COL1A1, COL1A2, IL2RB, PLA2G10, ANGPT2, PROCR, NOX4, HAMP, PTPN11, SLCA1, IL2RA, CCL5, IRF1, CFAR, CACA, EIF4E, GSTP1, JAK2, CYP3A5, HSPG2, CCL3, MYDSS, VIP, SOAT1, ADRBK1, NR4A2, MMPS, NPR2, GCH1, EPRS, PPARGC1A, F12, PECAM1, CCL4, CERPINA34, CASR, FABP2, TTF2, PROS1, CTF1, SGCB, YME1L1, CAMP, ZC3H12A, AKR1B1, MMP7, AHR, CSF1,

Disease(s)	Gene(s)
	HDAC9, CTGF, KCNMA1, UGT1A, PRKCA, COMT, S100B, EGR1, PRL, IL15, DRD4, CAMK2G, SLC22A2, CCL11, PGF, THPO, GP6, TACR1, NTS, HNF1A, SST, KCDN1, LOC646627, TBXAS1, CUP2J2, TBXA2R, ADH1C, ALOX12, AHSG, BHMT, GJA4, SLC25A4, ACLY, ALOX5AP, NUMA1, CYP27B1, CYSLTR2, SOD3, LTC4S, UCN, GHRL, APOC2, CLEC4A, KBTBD10, TNC, TYMS, SHC1, LRP1, SOCS3, ADH1B, KLK3, HSD11B1, VKORC1, SERPINB2, TNS1, RNF19A, EPOR, ITGAM, PITX2, MAPK7, FCGR3A, LEEPR, ENG, GPX1, GOT2, HRH1, NR112, CRH, HTR1A, VDAC1, HPSE, SFTPD, TAP2, RMF123, PTK2Bm NTRK2, IL6R, ACHE, GLP1R, GHR, GSR, NQO1, NR5A1, GJB2, SLC9A1, MAOA, PCSK9, FCGR2A, SERPINF1, EDN3, UCP2, TFAP2A, C4BPA, SERPINF2, TYMP, ALPP, CXCR2, SLC3A3, ABCG2, ADA, JAK3, HSPA1A, FASN, FGF1, F11, ATP7A, CR1, GFPA, ROCK1, MECP2, MYLK, BCHE, LIPE, ADORA1, WRN, CXCR3, CD81, SMAD7, LAMC2, MAP3K5, CHGA, IAPP, RHO, ENPP1, PTHLH, NRG1, VEGFC, ENPEP, CEBPB, NAGLU, F2RL3, CX3CL1, BDKRB1, ADAMTS13, ELANE, ENPP2, CISH, GAST, MYOC, ATP1A2, NF1, GJB1, MEF2A, VCL, BMPR2, TUBB, CDC42, KRT18, HSF1, MYB, PRKAA2, ROCK2, TFP1, PRKG1, BMP2, CTNND1, CTH, CTSS, VAV2, NPY2R, IGFBP2, CD28, GSTA1, PPIA, APOH, S100A8, IL11, ALOX15, FBLN1, NR1H3, SCD, GIP, CHGB, PRKCB, SRD5A1, HSD11B2, CALCRL, GALNT2, ANGPTL4, KCNN4, PIK3C2A, HBEGF, CYP7A1, HLA-DRB5, BNIP3, GCKR, S100A12, PADI4, HSPA14, CXCR1, H19, KRTAP19-3, IDDM2, RAC2, YRY1, CLOCK, NGFR, DBH, CHRNA4, CACNA1C, PRKAG2, CHAT, PTGDS, NR1H2, TEK, VEGFB, MEF2C, MAPKAPK2, TNFRSF11A, HSPA9, CYSLTR1, MAT1A, OPRL1, IMPA1, CLCN2, DLD, PSMA6, PSMBS, CHI3L1, ALDH1B1, PARP2, STAR, LBP, ABCC6, RGS2, EFNB2, GJB6, APOA2, AMPD1, DYSF, FDFT1, EMD2, CCR6, GJB3, IL1RL1, ENTPD1, BBS4, CELSR2, F11R, RAPGEF3, HYAL1, ZNF259, ATOX1, ATF6, KHK, SAT1, GGH, TIMP4, SLC4A4, PDE2A, PDE3B, FADS1, FADS2, TMSB4X, TXNIP, LIMS1, RHOB, LY96, FOXO1, PNPLA2, TRH, GJC1, SLC7A5, FTO, GJD2, PRSC1, CASP12, GPBAR1, PXX, IL33, TRIB1, PBX4, NUPR1, 15-SEP, CILP2, TERC, GGT2, MTCO1, UOX, AVP, ANGPTL3

Disease(s)	Gene(s)
Cataract	CRYAA, CRYA1, CRYBB2, CRYB2, PITX3, BFSP2, CP49, CP47, CRYAA, PAX6, AN2, MGDA, CRYBA1, CRYB1, CRYGC, CRYG3, CCL, LIM2, MP19, CRYGD, CRYG4, BFSP2, CP49, CP47, HSF4, CTM, MIP, AQP0, CRYAB, CRYA2, CTPP2, CRYBB1, CRYGD, CRYG4, CRYBB2, CRYB2, CCL, GJA8, CX50, CAE1, GJA3, CX46, CZP3, CAE3, CCM1, CAM, KRIT1
Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)	CASQ2
CDKL-5 Deficiencies or Mediated Diseases	CDKL5
Charcot-Marie-Tooth (CMT) disease (Types 1, 2, 3, and 4)	PMP22 (CMT1A and E), MPZ (CMT1B), LITAF (CMT1C), EGR2 (CMT1D), NEFL (CMT1F), GJB1 (CMT1X), MFN2 (CMT2A), KIF1B (CMT2A2B), RAB7A (CMT2B), TRPV4 (CMT2C), GARS (CMT2D), NEFL (CMT2E), GAPD1 (CMT2K), HSPB8 (CMT2L), DYNC1H1 (CMT2O), LRSAM1 (CMT2P), IGHMBP2 (CMT2S), MORC2 (CMT2Z), GDAP1 (CMT4A), MTMR2 or SBF2/MTMR13 (CMT4B), SH3TC2 (CMT4C), NDRG1 (CMT4D), PRX (CMT4F), FIG4 (CMT4J), NT-3
Chediak-Higashi Syndrome	LYST
Choroideremia	CHM, REP1
Chorioretinal Atrophy	PRDM13, RGR, TEAD1
Chronic Granulomatous Disease	CYBA, CYBB, NCF1, NCF2, NCF4
Chronic Heart Failure	SERCA2
Chronic Mucocutaneous Candidiasis	AIRE, CARD9, CLEC7A, IL12B, IL12B1, IL1F, IL17RA, IL17RC, RORC, STAT1, STAT3, TRAF31P2
Cirrhosis	KRT18, KRT8, CIRH1A, NAIC, TEX292, KIAA1988
Colon Cancer (Familial Adenomatous Polyposis (FAP) and Hereditary Nonpolyposis Colon Cancer (HNPCC))	FAP:APC, HNPCC: MSH2, MLH1, PMS2, SH6, PMS1
Combined Immunodeficiency	IL2RG, SCIDX1, SCIDX, IMD4, HIV-1 (CCL5, SCYA5, D17S136E, TCP228)
Cone(-rod) Dystrophy	AIPL1, CRX, GUA1A, GUCY2D, PITPM3, PROM1, PRPH2, RIMS1, SEMA4A, ABCA4, ADAM9, ATF6, C21ORF2, C8ORF37, CACNA2D4, CDHR1, CERKL, CNGA3, CNGB3, CNRM4, CNAT2, IFT81, KCNV2, PDE6C, PDE6H, POC1B, RAX2, RDH5, RPGRIP1, TTLL5, RetCG1, GUCY2E
Congenital Stationary Night Blindness	CABP4, CACNA1F, CACNA2D4, GNAT1, CPR179, GRK1, GRM6, LRIT3, NYX, PDE6B, RDH5, RHO, RLBP1, RPE65, SAG, SLC24A1, TRPM1
Congenital Fructose Intolerance	ALDOB
Cori's Disease (Glycogen Storage Disease Type III)	AGL
Corneal Clouding and Dystrophy	APOA1, TGFB1, CSD2, CDGG1, CSD, BIGH3, CDG2, TACSTD2, TROP2, M1S1, VSX1, RINX, PPCD, PPD, KTCN, COL8A2, FECD, PPCD2, PIP5K3, CFD
Cornea Plana Congenital	KERA, CNA2

Disease(s)	Gene(s)
Cri Du Chat Syndrome, also known as 5p Syndrome and Cat Cry Syndrome	Deletions involving only band 5p15.2 to the entire short arm of chromosome 5, e.g., CTNND2, TERT
Crigler-Najjar syndrome	UGT1A1
Cystic Fibrosis (CF)	CTFR, ABCC7, CF, MRP7, SCNN1A, those described in WO2015157070
Diabetic Nephropathy	Gremlin, 12/15- lipoxxygenase, TIM44
Dent Disease (Types 1 and 2)	Type 1: CLCN5, Type 2: ORCL
Dentatorubral-Pallidoluysian Atrophy (DRPLA) (aka Haw River and Naito-Oyanagi Disease)	Atrophin-1 and ATN1
Down Syndrome	Chromosome 21 trisomy
Drug Addiction	PRKCE, DRD2, DRD4, ABAT, GRIA2, GRM5, GRIN1, HTR1b, GRIN2A, DRD3, PDYN, GRIA1
Duane Syndrome (Types 1, 2, and 3, including subgroups A, B, and C). Other names for this condition include Duane's Retraction Syndrome (or DR Syndrome), Eye Retraction Syndrome, Retraction Syndrome, Congenital retraction Syndrome, and Stilling-Turk-Duane Syndrome	CHN1, indels on chromosomes 4 and 8
Duchenne Muscular Dystrophy (DMD)	DMD, BMD, dystrophin gene, intron flanking exon 51 of DMD gene, exon 51 mutations in DMD gene, see also WO2013163628 and US Pat. Pub. 20130145487
Eating Disorders	BDNF
Edward's Syndrome (Trisomy 18)	Complete or partial trisomy of chromosome 18
Ehlers-Danlos Syndrome (Types I-VI)	COL5A1, COL5A2, COL1A1, COL3A1, TNXB, PLOD1, COL1A2, FKBP14, ADAMTS2
Emery-Dreifuss Muscular Dystrophy	LMNA, LMN1, EMD2, FPLD, CMD1A, HGPS, LGMD1B, LMNA, LMN1, EMD2, FPLD, CMD1A
Enhanced S-Cone Syndrome	NR2E3, NRL
Epilepsy	NPY
Fabry's Disease	GLA, AGA
Facioscapulohumeral Muscular Dystrophy	FSHMD1A, FSHD1A, FRG1
Factor H and Factor H-like 1	HF1, CFH, HUS
Factor V Leiden Thrombophilia and Factor V Deficiency	Factor V (F5)
Factor V and Factor VII Deficiency	MCFD2
Factor VII Deficiency	F7
Factor X Deficiency	F10
Factor XI Deficiency	F11
Factor XII Deficiency	F12, HAF
Factor XIII A Deficiency	F13A1, F13A
Factor XIII B Deficiency	F13B
Familial Hypercholesterolemia	APOB, LDLR, PCSK9
Familial Mediterranean Fever (FMF) also called Recurrent Polyserositis or Familial Paroxysmal Polyserositis	MEFV
Fanconi Anemia	FANCA, FACA, FA1, FA, FAA, FAAP95, FAAP90, FLJ34064, FANCC, FANCG, RAD51, BRCA1, BRCA2, BRIP1, BACH1, FANCI, FANCB, FANCD1, FANCD2, FANCD, FAD, FANCE, FACE, FANCF, FANCI, ERCC4, FANCL, FANCM, PALB2, RAD51C, SLX4, UBE2T, FANCB, XRCC9, PHF9, KIAA1596
Fanconi Syndrome Types I (Childhood onset) and II (Adult Onset)	FRTS1, GATM
Fragile X Syndrome and related disorders	FMR1, FMR2, FXR1, FXR2, mGLUR5

Disease(s)	Gene(s)
Fragile XE Mental Retardation (aka Martin Bell Syndrome)	FMR1
Friedreich Ataxia (FRDA)	FXN/X25
Fuchs Endothelial Corneal Dystrophy	TCF4, COL8A2
Galactosemia	GALT, GALK1, GALE
Gastrointestinal Epithelial Cancer, GI cancer	CISH
Gaucher Disease (Types 1, 2, and 3, as well as other unusual forms that may not fit into these types)	GBA, GCase
Glaucoma	MYOC, TIGR, GLC1A, JOAG, GPOA, OPTN, GLC1E, FIP2, HYPL, NRP, CYP1B1, GLC3A, OPA1, NTG, NPG, CYP1B1, GLC3A, those described in WO2015153780
Glomerulosclerosis	CC chemokine ligand 2
Glycogen Storage Diseases (Types I-VI - See also Cori's Disease, Pompe Disease, McArdle's Disease, Hers Disease, and Von Gierke's disease)	SLC2A2, GLUT2, G6PC, G6PT, G6PT1, GAA, LAMP2, LAMPB, AGL, GDE, GBE1, GYS2, PYGL, PFKM, see also Cori's Disease, Pompe's Disease, McArdle's disease, Hers Disease, and Von Gierke's disease
RBC Glycolytic Enzyme Deficiency	any mutations in a gene for an enzyme in the glycolysis pathway including mutations in genes for hexokinases I and II, glucokinase, phosphoglucose isomerase, phosphofructokinase, aldolase Bm triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerokinase, phosphoglycerate mutase, enolase I, pyruvate kinase
GM2 Gangliosidosis (e.g., Sandhoff Disease and Tay-Sachs Disease)	Hexosaminidase-alpha, hexosaminidase-beta, HEX-A
Glycogen Storage Disease Type Ia (GSDIa)	G6Pase
Hemophilia (A or B)	F8, F9
Hartnup Disease	SLC6A19
Hearing Loss	NOX3, Hes5, BDNF
Hemochromatosis (HH)	HFE, H63D
Hemophagocytic Lymphohistiocytosis Disorders	PRF1, HPLH2, UNC13D, MUNC13-4, HPLH3, HLH3, FHL3
Hemorrhagic Disorders	PI, ATT, F5
Hers Disease (Glycogen Storage Disease Type VI)	PYGL
Hereditary Angioedema (HAE)	kalikrein B1
Hereditary Hemorrhagic Telangiectasia (Osler-Weber- Rendu Syndrome)	ACVRL1, ENG, SMAD4
Hereditary Spherocytosis	NK1, EPB42, SLC4A1, SPTA1, SPTB
Hereditary Persistence of Fetal Hemoglobin	HBG1, HBG2, BCL11A, promoter region of HBG 1 and/or 2 (in the CCAAT box)
Hemophilia (Hemophilia A (Classic), B (aka Christmas disease), and C)	A: FVIII, F8C, HEMA B: FVIX, HEME, FIX C: F9, F11
Hepatic Adenoma	TCF1, HNF1A, MODY3
Hepatic Failure, early onset, and Neurologic Disorder	SCOD1, SCO1
Hepatic Lipase Deficiency	LIPC
Hepatoblastoma, Cancer, and Carcinomas	CTNNB1, PDGFRL, PDGRL, PRLTS, AXIN1, AXIN, CTNNB1, TP53, P53, LFS1, IGF2R, MPR1, MET, CASP8, MCH5

Disease(s)	Gene(s)
Hermansky-Pudlak Syndrome	HPS1, HPS3, HPS4, HPS5, HPS6, HPS7, DTNBP1, BLOC1, BLOC1S2, BLOC3
Human immunodeficiency viruses (HIV) susceptibility or infection	IL10, CSIF, CMKBR2, CCR2, CMKBR5, CCKR5 (CCR5), those in WO2015148670A1
Holoprosencephaly (HPE) (Alobar, Semilobar, and Lobar)	ACVRL1, ENG, SMAD4
Homocystinuria	CBS, MTHFR, MTR, MTRR, MMADHC
Human papillomavirus (HPV)	HPV16, HPV18 E6/E7
Herpes simplex virus (HSV)1, HSV2, and related Keratitis	HSV1 genes (immediate early and late HSV-1 genes (UL1, 1.5, 5, 6, 8, 9, 12, 15, 16, 18, 19, 22, 23, 26, 26.5, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 42, 48, 49.5, 50, 52, 54, S6, RL2, RS1, those described in WO2015153789, WO2015153791
Hunter's Syndrome (aka Mucopolysaccharidosis Type II)	IDS
Huntington's Disease (HD) and HD-like Disorders	HD, HTT, IT15, PRNP, PRIP, JPH3, JP3, HDL2, TBP, SCA17, PRKCE, BDNF, IGF1, EP300, RCOR1, PRKCZ, HDAC4, TGM2, those described in WO2013130824, WO2015089354
Hurler's Syndrome (aka Mucopolysaccharidosis (MPS) Type IH, MPS IH)	IDUA, α -L-iduronidase
Hurler-Scheie Syndrome (aka Mucopolysaccharidosis Type IH-S, MPS IH-S)	IDUA, α -L-iduronidase
Hyaluronidase Deficiency (aka MPS IX)	HYAL1
Hypercholesterolaemia or Phenylketonuria (PKU)	PAH, LDLR
Hyper IgM Syndrome	CD40L
Hypertension caused Renal Damage	Mineral corticoid receptor
Immunodeficiencies	CD3E, CD3G, AICDA, AID, HIGM2, TNFRSF5, CD40, UNG, DGU, HIGM4, TNFSF5, CD40LG, HIGM1, IGM, FOXP3, IPEX, AIID, XPID, PIDX, TNFRSF14B, TACI
Inborn errors of metabolism: including urea cycle disorders, organic acidemias, fatty acid oxidation defects, amino acidopathies, carbohydrate disorders, mitochondrial disorders	See also: Carbohydrate Metabolism Disorders (e.g., Galactosemia), Amino Acid Metabolism Disorders (e.g., Phenylketonuria), Fatty Acid Metabolism (e.g., MCAD deficiency), Urea Cycle Disorders (e.g., Citrullinemia), Organic Acidemias (e.g., Maple Syrup Urine Disease), Mitochondrial Disorders (e.g., MELAS), Peroxisomal Disorders (e.g., Zellweger syndrome)
Inflammation	IL-10, IL-1 (IL-1a, IL-1b), IL-13, IL-17 (IL-17a (CTLA8), IL-17b, IL-17c, IL-17d, IL-17f), IL-23, Cx3crl, ptpn22, TNFa, NOD2/CARD15 for IBD, IL-6, IL-12 (IL-12a, IL-12b), CTLA4, Cx3c11
Inflammatory Bowel Diseases (e.g., Ulcerative Colitis and Cronh's Disease)	NOD2, IRGM, LRRK2, ATG5, ATG16L1, IRGM, GATM, ECM1, CDH1, LAMB1, HNF4A, GNA12, IL10, CARD9/15, CCR6, IL2RA, MST1, TNFSF15, REL, STAT3, IL23R, IL12B, FUT2
Interstitial Renal Fibrosis	TGF- β type II receptor
Job's Syndrome (aka Hyper IgE Svndrome)	STAT3, DOCK8
Juvenile Retinoschisis	RS1, XLR51
Kabuki Syndrome 1	MLL4, KMT2D

Disease(s)	Gene(s)
Kennedy Disease (aka Spinobulbar Muscular Atrophy)	SBMA/SMAX1/AR
Klinefelter Syndrome	Extra X chromosome in males
Lafora Disease	EMP2A, EMP2B
Leber Congenital Amaurosis	CRB1, RP12, CORD2, CRD, CRX, IMPDH1, OTX2, AIPL1, CABP4, CCT2, CEP290, CLUAP1, CRB1, CRX, DTHD1, GDF6, GUCY2D, IFT140, IQCB1, KCNJ13, LCA5, LRAT, NMNAT1, PRPH2, RD3, RDH12, RPE65, RP20, RPGRIP1, SPATA7, TULP1, LCA1, LCA4, GUC2D, CORD6, LCA3
Leber Hereditary Optic	ND4
Lesch-Nyhan Syndrome	HPRT1
Leukocyte deficiencies and disorders	ITGB2, CD18, LCAMB, LAD, EIF2B1, EIF2BA, EIF2B2, EIF2B3, EIF2B5, LVWM, CACH, CLE, EIF2B4
Leukemia	TAL1, TCL5, SCL, TAL2, FLT3, NBS1, NBS, ZNFN1A1, IK1, LYF1, HOXD4, HOX4B, BCR, CML, PHL, ALL, ARNT, KRAS2, RASK2, GMPS, AF10, ARHGEF12, LARG, KIAA0382, CALM, CLTH, CEBPA, CEBP, CHIC2, BTL, FLT3, KIT, PBT, LPP, NPM1, NUP214, D9S46E, CAN, CAIN, RUNX1, CBFA2, AML1, WHSC1L1, NSD3, FLT3, AF1Q, NPM1, NUMA1, ZNF145, PLZF, PML, MYL, STAT5B, AF10, CALM, CLTH, ARL11, ARLTS1, P2RX7, P2X7, BCR, CML, PHL, ALL, GRAF, NF1, VRNF, WSS, NFNS, PTPN11, PTP2C, SHP2, NS1, BCL2, CCND1, PRAD1, BCL1, TCRA, GATA1, GF1, ERYF1, NFE1, ABL1, NQO1, DIA4, NMOR1, NUP214, D9S46E, CAN, CAIN
Limb-Girdle Muscular Dystrophies	LGMD
Lipoprotein lipase (LPL) deficiency	LPL
Lowe Syndrome	OCRL
Lupus Glomerulonephritis	MAPK1
Lysosomal storage disorders	GDNF
Machado-Joseph's Disease (also known as Spinocerebellar Ataxia Type 3)	ATX3
Macular Degeneration	ABC4, CBC1, CHM1, APOE, C1QTNF5, C2, C3, CCL2, CCR2, CD36, CFB, CFH, CFHR1, CFHR3, CNGB3, CP, CRP, CST3, CTSD, CX3CR1, ELOVL4, ERCC6, FBLN5, FBLN6, FSCN2, HMCN1, HTRA1, IL6, IL8, PLEKHA1, PROM1, PRPH2, RPGR, SERPING1, TCOF1, TIMP3, TLR3
Macular Dystrophy	BEST1, C1QTNF5, CTNNA1, EFEMP1, ELOVL4, FSCN2, GUCA1B, HMCN1, IMPG1, OTX2, PRDM13, PROM1, PRPH2, RP1L1, TIMP3, ABCA4, CFH, DRAM2, IMG1, MFSD8, ADMD, STGD2, STGD3, RDS, RP7, PRPH, AVMD, AOFMD, VMD2
Malattia Leventinese	EFEMP1, FBLN3
Malignant Melanoma	CD86, B7-2, IL-12
Maple Syrup Urine Disease	BCKDHA, BCKDHB, DBT
Marfan Syndrome	FBN1
Maroteaux-Lamy Syndrome (aka MPS VI)	ARSB

Disease(s)	Gene(s)
medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	MCAD
McArdle's Disease (Glycogen Storage Disease Type V, myophosphorylase deficiency)	PYGM
Medullary Cystic Kidney Disease	UMOD, HNFJ, FJHN, MCKD2, ADMCKD2
Metachromatic Leukodystrophy	ARSA
Methylmalonic Acidemia (MMA)	MMAA, MMAB, MUT, MMACHC, MMADHC, LMBRD1
Mitochondrial Heteroplasmy, Myoclonic Epilepsy with ragged red fibers (MERRF), or Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes (ME LAS)	APAL1
Morquio Syndrome (aka MPS IVA and B)	GALNS
Mucopolysaccharidosis Type VII (MPSVII) or Sly Syndrome	GUSB
Mucopolysaccharidosis diseases (Types I H/S, I H, II, III A B and C, I S, IVA and B, IX, VII, and VI)	See also Hurler/Scheie Syndrome, Hurler Disease, Sanfilippo Syndrome, Scheie Syndrome, Morquio Syndrome, Hyaluronidase Deficiency, Sly Syndrome, and Maroteaux-Lamy Syndrome
Muscular Atrophy	VAPB, VAPC, ALS8, SMN1, SMA1, SMA2, SMA3, SMA4, BSCL2, SPG17, GARS, SMAD1, CMT2D, HEXB, IGHMBP2, SMUBP2, CATF1, SMARD1
Muscular Dystrophy	Sarcoglycan α , β , γ , Δ , ϵ , or ζ (SGCA, SGCB, SGCG, SGCD, SGCE, or SGCZ), FKRP, MDC1C, LGMD2I, LAMA2, LAMM, LARGE, KIAA0609, MDC1D, FCMD, TTID, MYOT, CAPN3, CANP3, DYSF, LGMD2B, LGMD2C, DMDA1, SCG3, ADL, DAG2, LGMD2D, DMDA2, LGMD2E, SGD, LGMD2F, CMD1L, TCAP, LGMD2G, CMD1N, TRIM32, HT2A, LGMD2H, FKRP, MDC1C, LGMD2I, TTN, CMD1G, TMD, LGMD2J, POMT1, CAV3, LGMD1C, SEPN1, SELN, RSMD1, PLEC1, PLTN, EBS1, dystrophin, mini-dystrophin
Myotonic Dystrophy (Type 1 and Type 2)	CNBP (Type 2), DMPK (Type 1)
Neoplasia	PTEN, ATM, ATR, EGFR, ERBB2, ERBB3, ERBB4, NOTCH1, NOTCH2, NOTCH3, NOTCH4, AKT, AKT2, AKT3, HIF, HIF1A, HIF3A, MET, HRG, BCL2, PPAR alpha, PPAR gamma, WT1 (Wilms Tumor), FGF Receptor Family members (5 members: 1, 2, 3, 4, 5), CDKN2A, APC, RB (retinoblastoma), MEN1, VHL, BRCA1, BRCA2, AR (Androgen Receptor), TSG101, IGF, IGF Receptor IGF1 (4 variants), IGF2 (3 variants), IGF1 Receptor, IGF 2 Receptor, BAX, BCL2, caspases family (9 members: 1, 2, 3, 4, 6, 7, 8, 9, 12), KRAS, APC
Neurofibromatosis (NF) (NF1, formerly Recklinghausen's NF, and NF2)	NF1, NF2
Niemann-Pick Lipidosis (Types A, B, and C)	Types A and B: SMPD1, Type C: NPC1 or NPC2
Noonan Syndrome	PTPN11, SOS1, RAF1, KRAS
Norrie Disease or X-linked Familial Exudative Vitreoretinopathy	NDP
North Carolina Macular Dystrophy	MCDR1
Ornithine Carbamoyltransferase Deficiency	OTC

Disease(s)	Gene(s)
Osteogenesis Imperfecta (OI) (Types I, II, III, IV, V, VI, and VII)	COL1A1, COL1A2, CRTAP, P3H
Osteopetrosis	LRP5, BMND1, LRP7, LR3, OPPG, VBCH2, CLCN7, CLC7, OPTA2, OSTM1, GL, TCIRG1, TIRC7, OC116, OPTB1
Patau's Syndrome (Trisomy 13)	Additional copy of chromosome 13
Parkinson's Disease (PD)	SNCA (PARK1), UCHL1 (PARK 5), and LRRK2 (PARK8), PARK3, PARK2, PARK4, PARK7, PINK1 (PARK6), x-Synuclein, DJ-1, Parkin, NR4A2, NURR1, NOT, TINUR, SNCAIP, TBP, SCA17, NCAP, PRKN, PDJ, DBH, NDUFV2, BDNF, GDNF, GBA, AADC, GAD1, GAD2, NRTN, NPY
Pattern Dystrophy of the retinal pigment epithelium (RPE)	RDS/peripherin
Phenylketonuria (PKU)	PAH, PKU1, QDPR, DHPR, PTS
Polycystic Kidney and Hepatic Disease	FCYT, PKHD1, ARPKD, PKD1, PKD2, PKD4, PKDTS, PRKCSH, G19P1, PCLD, SEC63
Pompe Disease	GAA
Porphyria (actually refers to a group of different diseases all having a specific heme production process abnormality)	ALAD, ALAS2, CPOX, FECH, HMBS, PPOX, UROD, UROS
Posterior Polymorphous Corneal Dystrophy	TCF4, COL8A2
Prader-Willi Syndrome	Deletion of region of short arm of chromosome 15, including UBE3A
Primary Hyperoxaluria (e.g., Type 1)	LDHA (lactate dehydrogenase A) and hydroxyacid oxidase 1 (HAO1)
Primary Open Angle Glaucoma (POAG)	MYOC
Primary Sclerosing Cholangitis	TCF4, COL8A2
Progeria (also called Hutchinson-Gilford Progeria Syndrome)	LMNA
Progressive External Ophthalmoplegia	ANT-1
Propionic Acidaemias	PCCA
Prostate Cancer	HOXB13, MSMB, GPRC6A, TP53, GM-CSF
Pyruvate Dehydrogenase Deficiency	PDHA1
Kidney/Renal Carcinoma	RLIP76, VEGF
Rett Syndrome	MECP2, RTT, PPMX, MRX16, MRX79, CDKL5, STK9, MECP2, RTT, PPMX, MRX16, MRX79, x-Synuclein, DJ-1

Disease(s)	Gene(s)
Retinitis Pigmentosa (RP)	ADIPOR1, ABCA4, AGBL5, ARHGEF18, ARL2BP, ARL3, ARL6, BEST1, BBS1, BBS2, C2ORF71, C8ORF37, CA4, CERKL, CLRN1, CNGA1, CMGB1, CRB1, CRX, CYP4V2, DHDDS, DHX38, EMC1, EYS, FAM161A, FSCN2, GPR125, GUCA1B, HK1, HPRPF3, HGSNAT, IDH3B, IMPDH1, IMPG2, IFT140, IFT172, KLHL7, KIAA1549, KIZ, LRAT, MAK, MERTK, MVK, NEK2, NUROD1, NR2E3, NRL, OFD1, PDE6A, PDE6B, PDE6G, POMGNT1, PRCD, PROM1, PRPF3, PRPF4, PRPF6, PRPF8, PRPF31, PRPH2, RPB3, RDH12, REEP6, RP39, RGR, RHO, RLBP1, ROM1, RP1, RP1L1, RPY, RP2, RP9, RPE65, RPGR, SAMD11, SAG, SEMA4A, SLC7A14, SNRNP200, SPP2, SPATA7, TRNT1, TOPORS, TTC8, TULP1, USH2A, ZNF408, ZNF513, see also 20120204282
Sanfilippo Syndrome or Mucopolysaccharidosis Type III B (MPS IIIB)	NAGLU
short-chain acyl-CoA dehydrogenase (SCAD) deficiency	SCAD
Scheie Syndrome (also known as Mucopolysaccharidosis Type I S (MPS I-S))	IDUA, α -L-iduronidase
Schizophrenia	Neuregulin1 (NRG1), ERB4 (receptor for Neuregulin), Complexin1 (CPLX1), TPH1 Tryptophan hydroxylase, TPH2 Tryptophan hydroxylase 2, Neurexin 1, GSK3, GSK3a, GSK3b, 5-HTT (Slc6a4), COMT, DRD (Drd1a), SLC6A3, DAOA, DTNBP1, Dao (Dao1), TCF4, COL8A2

Disease(s)	Gene(s)
Secretase Related Disorders	APH-1 (alpha and beta), PSEN1, NCSTN, PEN-2, Nos1, Parp1, Nat1, Nat2, CTSB, APP, APH1B, PSEN2, PSENEN, BACE1, ITM2B, CTSD, NOTCH1, TNF, INS, DYT10, ADAM17, APOE, ACE, STN, TP53, IL6, NGFR, IL1B, ACHE, CTNNA1, IGF1, IFNG, NRG1, CASP3, MAPK1, CDH1, APBB1, HMGCR, CREB1, PTGS2, HES1, CAT, TGFB1, ENO2, ERBB4, TRAPPC10, MAOB, NGF, MMP12, JAG1, CD40LG, PPARG, FGF2, LRP1, NOTCH4, MAPK8, PREP, NOTCH3, PRNP, CTSG, EGF, REN, CD44, SELP, GHR, ADCYAP1, INSR, GFAP, MMP3, MAPK10, SP1, MYC, CTSE, PPARA, JUN, TIMP1, IL5, IL1A, MMP9, HTR4, HSPG2, KRAS, CYCS, SMG1, IL1R1, PROK1, MAPK3, NTRK1, IL13, MME, TKT, CXCR2, CHRM1, ATXN1, PAWR, NOTCH2, M6PR, CYP46A1, CSNK1D, MAPK14, PRG2, PRKCA, L1 CAM, CD40, NR1H2, JAG2, CTNND1, CMA1, SORT1, DLK1, THEM4, JUP, CD46, CCL11, CAV3, RNASE3, HSPA8, CASP9, CYP3A4, CCR3, TFAP2A, SCP2, CDK4, JOF1A, TCF7L2, B3GALT1, MDM2, RELA, CASP7, IDE, FANP4, CASK, ADCYAP1R1, ATF4, PDGFA, C21ORF33, SCG5, RMF123, NKFB1, ERBB2, CAV1, MMP7, TGFA, RXRA, STX1A, PSMC4, P2RY2, TNFRSF21, DLG1, NUMBL, SPN, PLSCR1, UBQLN2, UBQLN1, PCSK7, SPON1, SILV, QPCT, HESS, GCC1
Selective IgA Deficiency	Type 1: MSH5, Type 2: TNFRSF13B
Severe Combined Immunodeficiency (SCID), SCID-XI, and Adenosine deaminase (ADA)-SCID	JAK3, JAK1, DCLRE1C, ARTEMIS, SCIDA, RAG1, RAG2, ADA, PTPRC, CD45, LCA, IL7R, CD3D, T3D, IL2RG, SCIDX1, SCIDX, IMD4, those identified in US Pat. App. Pub. 20110225664, 20110091441, 20100229252, 20090271881 and 20090222937
Sickle Cell Disease	HBB, BCL11A, BCL11Ae, cis- regulatory elements of the B-globin locus, HBG 1/2 promoter, HBG distal CCAAT box region between -92 and -130 of the HBG Transcription Start Site, those described in WO2015148863, WO 2013/126794, US Pat. Pub. 20110182867
Spinocerebellar Ataxias (SCA Types 1, 2, 3, 6, 7, 8, 12, and 17)	ATXN1, ATXN2, ATX3
Sorsby Fundus Dystrophy	TIMP3
Stargardt Disease	ABCR, ELOVL4, ABCA4, PROM1
Thalassemia (Alpha, Beta, Delta)	HBA1, HBA2 (Alpha), HBB (Beta), HBB and HBD (delta), LCRB, BCL11A, BCL11Ae, cis-regulatory elements of the B-globin locus, HBG 1/2 promoter, those described in WO2015148860, US Pat. Pub. 20110182867, 2015/148860
Thymic Aplasia (DiGeorge Syndrome; 22q 11.2 Deletion Syndrome)	Deletion of 30 to 40 genes in the middle of chromosome 22 at a location known as 22q11.2, including TBX1, DGCR8
Transthyretin Amyloidosis (ATTR)	TTR (transthyretin)
Trimethylaminuria	FMO3

Disease(s)	Gene(s)
Trinucleotide Repeat Disorders (generally)	HTT, SBMA/SMAX1/AR, FXN/X25 ATX3, ATXN1, ATXN2, DMPK, Atrophin-1 and Atn1 (DRPLA Dx), CBP (Creb-BP - global instability), VLDLR, Atxn7, Atxn10, FEN1, TNRC6A, PABPN1, JPH3, MED15, ATXN3, TBP, CACNA1A, ATXN80S, PPP2R2B, ATXN7, TNRC6B, TNRC6C, CELF3, MAB21L1, MSH2, TMEM185A, SIX5, CNPY3, RAXE, GNB2, RPL14, ATXN8, ISR, TTR, EP400, GIGYF2, OGG1, STC1, CNDP1, C10ORF2, MAML3, DKC1, PAXIP1, CASK, MAPT, SP1, POLG, AFF2, THBS1, TP53, ESR1, CGGBP1, ABT1, KLK3, PRNP, JUN, KCNN3, BAX, FRAXA, KBTBD10, MBNL1, RAD51, NCOA3, ERDA1, TSC1, COMP, GGLC, RRAD, MSH3, DRD2, CD44, CTCF, CCND1, CLSPN, MEF2A, PTPRU, GAPDH, TRIM22, WT1, AHR, GPX1, TPMT, NDP, ARX, TYR, EGR1, UNG, NUMBL, FABP2, EN2, CRYGC, SRP14, CRYGB, PDCD1, HOXA1, ATXN2L, PMS2, GLA, CBL, FTH1, IL12RB2, OTX2, HOXA5, POLG2, DLX2, AHRR, MANF, RMEM158, see also 20110016540
Triple X (XXX) Syndrome	X chromosome trisomy
Turner's Syndrome (XO)	Monosomy X
Tuberous Sclerosis	TSC1, TSC2
Usher Syndrome (Types I, II, and III)	ABHD12, CDH23, CIB2, CLRN1, DFNB31, GPR98, HARS, MYO7A, PCDH15, USH1C, USH1G, USH2A, USH11A, those described in WO2015134812A1
Velocardiofacial Syndrome (aka 22q11.2 Deletion Syndrome, DiGeorge Syndrome, Conotruncal Anomaly Face Syndrome (CTAF), Autosomal Dominant Opitz G/BB Syndrome or Cayler Cardio-Facial Syndrome)	Many genes are deleted, COM, TBX1, and other are associated with symptoms
very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency	VLCAD
Von Gierke's Disease (Glycogen Storage Disease Type I)	G6PC, SLC37A4
Von Hippel-Lindau Syndrome	VHL
Von Willebrand Disease (Types I, II, and III)	VWF
Wilson Disease	ATP7B
Wiskott-Aldrich Syndrome	WAS
Xeroderma Pigmentosum	POLH
X-linked myotubular myopathy	MTM1

A. Heterologous Expression of a Transgene for a Method of Treatment

- In some embodiments, a subject is administered an effective amount of a polypeptide comprising a transgene for expression of a protein of interest or a fragment thereof. In preferred embodiments, the polypeptide comprising a transgene is administered to the subject with a delivery vehicle such as a viral genome or an AAV vector (e.g., an rAAV vector).

In some embodiments, the method of treatment is intended to treat a disease or condition caused by a defect or deficiency in a single gene or single gene product (e.g., an mRNA transcript or

a protein). In some embodiments, the method of treatment is intended to treat a disease or condition that leads to a defect or deficiency in multiple gene products. In some embodiments, the defect or deficiency is defined by reduced expression, reduced activity, and/or aberrant localization of the one or more gene products (e.g., an mRNA transcript or a protein).

5 In some embodiments, the method of treatment is intended to replace, supplement, or replenish an absent, deficient (e.g., low expression levels), or defective (e.g., mutant, loss-of-function, or low biological or catalytic activity) gene product (e.g., an mRNA transcript or a protein) in a subject having a disorder or condition. In some embodiments, the disorder or condition is characterized by a loss-of-function mutation or a gene deletion. In other embodiments, the disorder or condition is
10 acquired (e.g., a deficient or defective gene product from stochastic or environmental factors).

In some embodiments, the transgene encodes a protein or fragment thereof that is identical (i.e., retains 100% sequence identity) to the wild-type amino acid sequence of the protein to replace, supplement, or replenish low levels a deficient or defective protein. In some embodiments, the transgene encodes a protein or fragment thereof that shares at least 85% (e.g., 85%, 86%, 87%,
15 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the wild-type amino acid sequence or a region therein to express a protein or fragment thereof that has improved biological function (e.g., increased catalytic function or reduced immunogenicity). Some examples in which a polypeptide or protein may be modified for improved biological function include mutating or adding sites for post-translational modifications (e.g., glycosylation, phosphorylation,
20 ubiquitination, nitrosylation, methylation, acetylation, lipidation, among others, or a combination thereof), mutating or adding cysteines for altered or added disulfide bonds, modifying binding sites for enhanced binding activity between the protein of interest and one or more known binding partners, modifying sites of protease binding or targeted cleavage, modifying a signal sequence for enhanced secretion or altered localization, among other protein modifications known in the art.

25 In some embodiments, the method of treatment is intended to increase or supplement the expression of a normally expressing protein (e.g., a protein that is present at a concentration within an accepted healthy range) in a subject having a disorder or condition that would benefit from increased expression of said protein. Increased expression of a normally expressing protein may be desired in order to increase the rate of an enzymatic reaction, enhance the potency or rate of a signaling
30 response (e.g., intracellularly or extracellularly), modulate trafficking or adhesion of a cell or cellular component, increase the likelihood or propensity of binding or a transient interaction to occur (e.g., based on the affinity or K_D of two or more molecules), or otherwise modulate one or more biological processes. Such methods may be clinically useful for increasing the expression of a protein with a redundant function to a deficient or defective protein. Such methods may also be clinically useful for
35 modulating a disease-causing protein or protein fragment that is logistically more difficult to employ for a method of treatment due to an assortment of non-limiting factors including large transgene size, low accessibility of a target cell or tissue, and/or high immunogenicity of the protein or fragment thereof.

In some embodiments, the method of treatment increases the expression of a protein or fragment thereof (e.g., upregulates, induces the expression of an exogenous protein or polypeptide)
40 to modulate or regulate a separate causative agent underlying a disease. Such embodiments may be

clinically useful for blocking, inhibiting, proteolyzing, mediating clearance of, or otherwise attenuating the effect of a causative agent such as a pathogen (e.g., a virus, a bacterium, a fungus, or a parasite) or a pro-inflammatory protein (e.g., a cytokine or a cytokine receptor).

In some embodiments, treatment with an effective amount of a polynucleotide comprising a transgene that encodes a protein or a pharmaceutical composition containing the same increases the expression of the gene by about 5% to 50% (e.g., about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%), by about 50% to 100% (about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%), or greater than 100% (about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300%, or more) as compared to a reference such as a biological sample from a control subject or a biological sample of the same subject prior to the administration of treatment. In some embodiments, a control subject is a subject with the same disease or condition who has not received the method of treatment. In other embodiments, a control subject is a healthy control subject.

In some embodiments, treatment with an effective amount of a polynucleotide comprising a transgene that encodes a protein or a pharmaceutical composition containing the same increases the expression of the gene by about 1-fold, by about 2-fold, by about 3-fold, by about 4-fold, by about 5-fold, by about 6-fold, by about 7-fold, by about 8-fold, by about 9-fold, by about 10-fold, by about 11-fold, by about 12-fold, by about 13-fold, by about 14-fold, by about 15-fold, by about 16-fold, by about 17-fold, by about 18-fold, by about 19-fold, by about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about 45-fold, about 50-fold, or more as compared to a reference such as a biological sample from a control subject or a biological sample of the same subject prior to the administration of treatment. In some embodiments, a control subject is a subject with the same disease or condition who has not received the method of treatment. In other embodiments, a control subject is a healthy control subject.

In some embodiments, an effective amount of a polynucleotide comprising a transgene that encodes a protein or a pharmaceutical composition containing the same corresponds to a therapeutic level of gene expression. In some embodiments, a therapeutic level is a physiologically acceptable expression level based on a healthy control subject or a median expression level based on a plurality of healthy control subjects. In some embodiments, a therapeutic level is an expression level that falls within a range of accepted expression levels based on subject characteristics (e.g., a subject's age, sex, weight, height, ethnicity, lifestyle, and/or one or more comorbidities). In further embodiments, a therapeutic level is an expression level that exceeds physiological values, such that the therapeutic expression level has about a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 425%, 450%, 475%, 500%, or more than about 500% greater relative to an expression level based on a healthy control subject or a median expression level based on a plurality of healthy control subjects.

In some embodiments, an effective amount of a polynucleotide comprising a transgene that encodes a protein or a pharmaceutical composition containing the same corresponds to a sub-

therapeutic level of gene expression. In some embodiments, a sub-therapeutic level is an expression level below a therapeutic level, such as an expression level below a physiologically acceptable expression level based on a healthy control subject or a median expression level based on a plurality of healthy control subjects. In some embodiments, a sub-therapeutic level is an expression level that falls within a range of accepted expression levels based on subject characteristics (e.g., a subject's age, sex, weight, height, ethnicity, lifestyle, and/or one or more comorbidities). In some embodiments, a sub-therapeutic level is an expression level that is below a physiological value, such that the sub-therapeutic expression level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% lower than a physiological value. In other embodiments, a sub-therapeutic level is an expression level that is below a therapeutic level, such that the sub-therapeutic expression level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% lower than a therapeutic level.

In some embodiments, treatment with an effective amount of a polynucleotide comprising a transgene that encodes a protein or a pharmaceutical composition containing the same results in ubiquitously increased expression of the gene, such there is a measurable increase across all organ systems or all tissues of the subject (e.g. throughout the body). In other embodiments, treatment with an effective amount of a polynucleotide comprising a transgene that encodes a protein or a pharmaceutical composition containing the same results in locally increased gene expression, such that there is a measurable increase in a limited region of the body of the subject (e.g., in a subset of tissues, in one or more organs, or in one or more organ system of interest).

In some embodiments, the increased expression of the gene following the treatment is measured by an increase in mRNA transcript levels or concentrations (e.g., an mRNA transcript that corresponds to the transgene) relative to a reference. Methods of measuring mRNA transcript expression levels are routine in the art. Exemplary methods of measuring mRNA transcript expression levels include, but are not limited to, quantitative polymerase chain reaction (qPCR), reverse transcription qPCR (RT-qPCR), RNA sequencing (RNA-seq), spectrophotometric analyses, flow cytometry, *in situ* hybridization methods, Northern blot analyses, or an array using one or more hybridizing oligonucleotides or antibodies against one or more mRNA transcripts of interest.

In some embodiments, the increased expression of the gene following the treatment is measured by an increase in protein expression levels or concentrations (e.g., a protein encoded by the transgene) relative to a reference. Methods of measuring protein expression levels are routine in the art. Exemplary methods of measuring protein expression levels include, but are not limited to, Western blot analysis, an enzyme-linked immunosorbent assay (ELISA), mass spectrometry, flow cytometry, fluorimetry, colorimetry, spectrophotometric analyses, or an array using a binding oligonucleotide (e.g., an aptamer) or an antibody against one or more protein of interest.

In some embodiments, the increased expression of the transgene that encodes a protein is sustained following administration. In some embodiments, the increased expression is sustained indefinitely following administration. In some embodiments, the increased expression is sustained for at least one week following administration. In some embodiments, the increased expression is sustained between one week and 10 years following administration. In some embodiments, the

increased expression is sustained between 1 week and 7 years following administration. In some embodiments, the increased expression is sustained between 1 week and 5 years following administration. In some embodiments, the increased expression is sustained between 1 week and 3 years following administration. In some embodiments, the increased expression is sustained between 1 week and 1 year following administration.

[illegible]

In some embodiments, the increased expression is sustained for more than 10 years following administration. In some embodiments, the increased expression is sustained between 6 months and 10 years following administration. In some embodiments, the increased expression is sustained between 1 year and 10 years following administration. In some embodiments, the increased expression is sustained between 2 years and 10 years following administration. In some

embodiments, the increased expression is sustained between 3 years and 10 years following administration. In some embodiments, the increased expression is sustained between 4 years and 10 years following administration. In some embodiments, the increased expression is sustained between 4 years and 10 years following administration. In some embodiments, the increased expression is sustained between 5 years and 10 years following administration. In some embodiments, the increased expression is sustained between 6 years and 10 years following administration. In some embodiments, the increased expression is sustained between 7 years and 10 years following administration. In some embodiments, the increased expression is sustained between 8 years and 10 years following administration. In some embodiments, the increased expression is sustained between 9 years and 10 years following administration.

The magnitude or duration of the increased expression of the mRNA transcript and/or protein encoded by the transgene following administration of the polynucleotide or pharmaceutical composition comprising the same may depend on one or more factors. Such factors may include, but are not limited to, the route of administration, the dose, stability of the mRNA transcript and/or protein, or the disease or condition for which the treatment is intended, and/or one or more comorbidities of the subject.

As described in the foregoing section, in some embodiments, the magnitude or duration of the increased expression of the mRNA transcript and/or protein encoded by the transgene is further modulated by administration of one or more inhibitory nucleic acid molecules (e.g., one or more inhibitory RNA molecules).

In some embodiments, the method of treatment includes administering an effective amount of a polynucleotide comprising a transgene or a pharmaceutical composition containing the same to the subject (e.g., a human) via *in vivo*, *in vitro*, or *ex vivo* methods of administration, or any combination thereof. In some embodiments, the method of treatment includes *in vivo* methods of administration, such that the polynucleotide or the pharmaceutical composition is administered directly to the body of the subject by an appropriate route of administration, such as one or more of the methods described below. In some embodiments, the method of treatment includes *in vitro* or *ex vivo* methods of administration, such that the polynucleotide or pharmaceutical composition containing the same is delivered to one or more isolated cells or tissues from the subject and then optionally separately implanted, dispensed, or deposited to the body of the subject.

The method of treatment includes administering an effective amount of a polynucleotide comprising a transgene for expression of a protein of interest or a fragment thereof or a pharmaceutical composition containing the same to the subject (e.g., a human) by any appropriate route of administration. Appropriate routes of administration include, but are not limited to, intramuscular, subcutaneous, intradermal, intravenous, intraperitoneal, intraarterial, intraventricular, intranasal, intraorbital, intracranial, or intraosseous administration. In some embodiments, the method of treatment includes administering one codon optimized gene product or a pharmaceutical composition containing the same to a subject to produce a therapeutic effect.

In some embodiments, an effective amount of a polypeptide comprising a transgene or a pharmaceutical composition containing the same is administered to a subject between 1 and 10

times. In some embodiments, an effective amount of a polypeptide comprising a transgene or a composition containing the same is administered to a subject between 1 and 5 times. In further embodiments, an effective amount of a polypeptide comprising a transgene or a pharmaceutical composition containing the same is administered to a subject between 3 and 5 times. In further
 5 embodiments, an effective amount of a polypeptide comprising a transgene or a pharmaceutical composition containing the same is administered to a subject between 1 and 3 times.

B. Reducing Heterologous Expression of a Transgene for a Method of Treatment

Following the administration of an effective amount of one or more polynucleotides
 10 comprising a transgene or one or more pharmaceutical compositions containing the same, it may be desirable to reduce expression of the transgene. Reducing expression may refer to dampening, lessening, or minimizing the potency of the polynucleotide comprising the transgene. For example, a subject that has been administered a polynucleotide comprising a transgene or a pharmaceutical composition containing the same may have a contraindication for which it is desirable to temporarily
 15 reduce expression of the transgene. Reducing expression of the transgene may be useful or recommended to a subject by a skilled practitioner (e.g., a physician or a clinician) for potential contraindications such as an acute infection, a surgical procedure, treatment with cytotoxic or chemotherapeutic agents, or fertility treatments and/or pregnancy of the subject.

Reducing the expression of a polynucleotide comprising a transgene or a pharmaceutical
 20 composition containing the same may be accomplished by administering one or more inhibitory nucleic acids (e.g. one or more inhibitory nucleic acids; e.g., one or more unmodified or modified inhibitory nucleic acids) or one or more pharmaceutical compositions containing the same, as described herein.

One or more inhibitory nucleic acids or pharmaceutical compositions containing the same
 25 may be administered to a subject to reduce the expression of the polynucleotide comprising the transgene temporarily or transiently. In some embodiments, an inhibitory nucleic acid or pharmaceutical composition containing the same is administered as a single administration. In some embodiments, an inhibitory nucleic acid or pharmaceutical composition containing the same is administered as a plurality of administrations.

An inhibitory nucleic acid may be administered to a subject at any time following
 30 administration of a polynucleotide comprising a transgene. In some embodiments, the inhibitory nucleic acid is administered at least one week following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between one week and 10 years following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered
 35 between 1 week and 7 years following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 5 years following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 3 years following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 1 year following administration of the polynucleotide.

40 In some embodiments, the inhibitory nucleic acid is administered between 1 week and 24

months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 23 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 22 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 21 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 20 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 19 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 18 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 17 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 16 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 15 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 14 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 13 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 12 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 11 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 10 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 9 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 8 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 7 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 6 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 5 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 4 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 3 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 2 months following administration of the polynucleotide. In some embodiments, the inhibitory nucleic acid is administered between 1 week and 1 month following administration of the polynucleotide.

In some embodiments, treatment with an effective amount of an inhibitory nucleic acid or a pharmaceutical composition containing the same reduces the expression of the gene (e.g., the transgene) by about 5% to 50% (e.g., about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%), by about 50% to 100% (about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%), or in some instances, greater than 100%, as compared to a reference

such as a biological sample from a control subject or a biological sample of the same subject prior to the administration of the treatment. In some embodiments, a control subject is a subject with the same disease or condition who has not received the method of treatment. In other embodiments, a control subject is a healthy control subject.

5 In some embodiments, treatment with an effective amount of an inhibitory nucleic acid or a pharmaceutical composition containing the same reduces the expression of the gene (e.g., the transgene) by about 1-fold, by about 2-fold, by about 3-fold, by about 4-fold, by about 5-fold, by about 6-fold, by about 7-fold, by about 8-fold, by about 9-fold, by about 10-fold, by about 11-fold, by about 12-fold, by about 13-fold, by about 14-fold, by about 15-fold, by about 16-fold, by about 17-fold, by
10 about 18-fold, by about 19-fold, by about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about 45-fold, about 50-fold, or more as compared to a reference such as a biological sample from a control subject or a biological sample of the same subject prior to the administration of treatment. In some embodiments, a control subject is a subject with the same disease or condition who has not received the method of treatment. In other embodiments, a control subject is a healthy
15 control subject.

In some embodiments, an effective amount of an inhibitory nucleic acid or a pharmaceutical composition containing the same corresponds to a therapeutic level of gene expression. In some embodiments, a therapeutic level is an expression level that is less than the level of gene expression following administration of a polynucleotide comprising a transgene, such that the therapeutic
20 expression level has about a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, or more than 100%, where applicable, relative the level of gene expression following administration of the polynucleotide comprising a transgene. In some embodiments, a therapeutic level is a physiologically acceptable expression level based on a healthy control subject or a median expression level based on a plurality of healthy control subjects.

25 In some embodiments, a therapeutic level is an expression level that falls within a range of accepted expression levels based on subject characteristics (e.g., a subject's age, sex, weight, height, ethnicity, lifestyle, and/or one or more comorbidities).

In some embodiments, an effective amount of an inhibitory nucleic acid or a pharmaceutical composition containing the same corresponds to a sub-therapeutic level of gene expression. In some
30 embodiments, a sub-therapeutic level is an expression level that is less than the therapeutic level, such that the sub-therapeutic expression level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% lower than a therapeutic level. In some embodiments, a sub-therapeutic level is an expression level that falls within a range of accepted expression levels based on subject characteristics (e.g., a subject's age, sex, weight, height, ethnicity,
35 lifestyle, and/or one or more comorbidities). In some embodiments, a therapeutic level is a physiologically acceptable expression level based on a healthy control subject or a median expression level based on a plurality of healthy control subjects. In some embodiments, a sub-therapeutic level is an expression level that is below a physiological value, such that the sub-therapeutic expression level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%,
40 60%, 65%, 70%, 75%, 80%, 85%, or 90% lower than a physiological value.

In further embodiments, an effective amount of an inhibitory molecule or a pharmaceutical composition containing the same results in a gene expression level that is comparable to a gene expression level measured in a sample obtained from the subject prior to administration of the polynucleotide comprising the transgene (i.e., levels prior to heterologous expression of the transgene).

In some embodiments, treatment with an effective amount of an inhibitory nucleic acid or a pharmaceutical composition containing the same results in ubiquitously reduced expression of the gene (e.g., the transgene), such that there is a measurable reduction in the gene across all organ systems or all tissues of the subject (e.g. throughout the body). In other embodiments, treatment with an effective amount of an inhibitory nucleic acid or a pharmaceutical composition containing the same results in locally reduced expression of the gene (e.g., the transgene), such that there is a measurable decrease in a limited region of the body (e.g., in a subset of tissues, in one or more organs, or in one or more organ system of interest).

In some embodiments, the decreased expression of the transgene following the treatment is measured by an decrease in mRNA transcript levels or concentrations (e.g., an mRNA transcript that corresponds to the transgene) relative to a reference. Methods of measuring mRNA transcript expression levels are routine in the art. Exemplary methods of measuring mRNA transcript expression levels include, but are not limited to, quantitative polymerase chain reaction (qPCR), reverse transcription qPCR (RT-qPCR), RNA sequencing (RNA-seq), spectrophotometric analyses, flow cytometry, *in situ* hybridization methods, Northern blot analyses, or an array using one or more hybridizing oligonucleotides or antibodies against one or more mRNA transcripts of interest.

In some embodiments, the reduced expression of the transgene following the treatment is measured by a reduction in protein expression levels or concentrations (e.g., a protein encoded by the transgene) relative to a reference. Methods of measuring protein expression levels are routine in the art. Exemplary methods of measuring protein expression levels include, but are not limited to, Western blot analysis, an enzyme-linked immunosorbent assay (ELISA), mass spectrometry, flow cytometry, fluorimetry, colorimetry, spectrophotometric analyses, or an array using a binding oligonucleotide (e.g., an aptamer) or an antibody against one or more protein of interest.

In some embodiments, the reduced expression of the transgene that encodes a protein is sustained for a given amount of time following administration. In some embodiments, the reduced expression is sustained for at least one week following administration. In some embodiments, the reduced expression is sustained between one week and 10 years following administration. In some embodiments, the reduced expression is sustained between 1 week and 9 years following administration. In some embodiments, the reduced expression is sustained between 1 week and 8 years following administration. In some embodiments, the reduced expression is sustained between 1 week and 7 years following administration. In some embodiments, the reduced expression is sustained between 1 week and 6 years following administration. In some embodiments, the reduced expression is sustained between 1 week and 5 years following administration. In some embodiments, the reduced expression is sustained between 1 week and 4 years following administration. In some embodiments, the reduced expression is sustained between 1 week and 3 years following

administration. In some embodiments, the reduced expression is sustained between 1 week and 2 years following administration. In some embodiments, the reduced expression is sustained between 1 week and 1 year following administration.

[illegible]

In some embodiments, the reduced expression is sustained for about 5 years following administration. In some embodiments, the reduced expression is sustained between 6 months and 5 years following administration. In some embodiments, the reduced expression is sustained between 1 year and 5 years following administration. In some embodiments, the reduced expression is sustained between 2 years and 5 years following administration. In some embodiments, the reduced expression is sustained between 3 years and 5 years following administration. In some embodiments, the reduced expression is sustained between 4 years and 5 years following administration.

40 The magnitude or duration of the reduced expression of the mRNA transcript and/or protein

encoded by the transgene following administration of the inhibitory nucleic acid or pharmaceutical composition containing the same may depend on one or more factors. Such factors may include, but are not limited to, the route of administration, the dose, stability of the mRNA transcript and/or protein, or the disease or condition for which the treatment is intended, and/or one or more comorbidities of the subject.

In some embodiments, the method of treatment includes delivering an effective amount of a polynucleotide comprising a transgene for expression of a protein of interest or a fragment thereof or a pharmaceutical composition containing the same via *in vivo*, *in vitro*, or *ex vivo* methods of delivery, or any combination thereof. In some embodiments, the method of treatment includes *in vivo* methods of administration, such that the polynucleotide or the pharmaceutical composition is administered directly to the body of the subject. In some embodiments, the method of treatment includes *in vitro* or *ex vivo* methods of administration, such that the polynucleotide or pharmaceutical composition containing the same is delivered to one or more isolated cells or tissues from the subject and then separately implanted, dispensed, or deposited to the body of the subject.

The method of treatment includes administering an effective amount of an inhibitory nucleic acid or a pharmaceutical composition containing the same to the subject (e.g., a human) by any appropriate route of administration. Appropriate routes of administration include, but are not limited to, intramuscular, subcutaneous, intradermal, intravenous, intraperitoneal, intraarterial, intraventricular, intranasal, intraorbital, intracranial, or intraosseous administration. In some embodiments, the method of treatment includes administering one codon optimized gene product or a pharmaceutical composition containing the same to a subject to produce a therapeutic effect.

In preferred embodiments, the route of administration of a polynucleotide comprising the transgene and an inhibitory nucleic acid or pharmaceutical compositions thereof are the same. In further embodiments, a polynucleotide comprising a transgene and an inhibitory nucleic acid or pharmaceutical compositions thereof are administered within a certain distance of a region of the body of a subject in need thereof (e.g., a human). In some embodiments, a polynucleotide comprising a transgene and an inhibitory nucleic acid or pharmaceutical compositions thereof are administered within a 10 cm distance or closer (e.g., within a 10 cm distance, within a 9 cm distance, within an 8 cm distance, within a 7 cm distance, within a 6 cm distance, within a 5 cm distance, within a 4 cm distance, within a 3 cm distance, within a 2 cm distance, within a 1 cm distance). In some embodiments, a polynucleotide comprising a transgene and an inhibitory nucleic acid or pharmaceutical compositions thereof are administered within a 3 cm distance or closer (e.g., within a 3 cm distance, within a 2 cm distance, within a 1 cm distance). In further embodiments, a polynucleotide comprising a transgene and an inhibitory nucleic acid or pharmaceutical compositions thereof are administered within 1 cm (e.g., within 1 cm, within 0.9 cm, within 0.8 cm, within 0.7 cm, within 0.6 cm, within 0.5 cm, within 0.4 cm, within 0.3 cm, or closer). In some embodiments, the location of the body of the subject for administration of a polynucleotide comprising a transgene and an inhibitory nucleic acid or pharmaceutical compositions comprising the same are marked prior to administration, e.g., by a medical tattoo.

V. Kits

The compositions or methods described herein can be provided in a kit for use in expressing a transgene and/or subsequently reducing the expression. In some embodiments, the compositions and methods described herein can be provided in a kit for use in treating a disease or condition. In some embodiments, the kit may include a package insert that instructs a user of the kit to construct or synthesize a polynucleotide comprising a transgene, a vector comprising the same, an inhibitory nucleic acid, or a pharmaceutical composition described herein. In further embodiments, the kit may include a package insert that instructs a user of the kit to perform any one of the methods of treatment described herein. The kit may optionally include a syringe or device for administering the compositions of the present disclosure. In some embodiments, the kit may include one or more additional therapeutic agents. In some embodiments, the kit includes one or more antibodies or binding molecules to detect the expression or activity of an mRNA transcript or a protein of interest.

EXAMPLES

15 **Example 1: Intramuscular administration of an siRNA reduces expression of a transgene delivered with an AAV vector**

This example describes the design and intramuscular administration of siRNA molecules for reducing expression of a transgene of interest following administration of a transgene with a designed AAV vector. A model AAV genome in an AAV9 serotype was designed to have multiple potential siRNA target sites in the 3'UTR of a transgene (FIG. 1), in which the potential siRNA target sites did not have any sequence identity to a region in a host genome (e.g., a mouse genome; e.g., a human genome). The transgene (e.g., the therapeutic transgene) was cyno dulaglutide (CyDula), a GLP-1 agonist protein that resembles dulaglutide but is modified to reduce immunogenicity in cynomolgus macaques.

25 The siRNA target site region in the transgene contained 7 distinct non-overlapping siRNA target sequences. For the proof-of-concept experiment, a single siRNA target sequence was chosen. Three siRNAs targeting the chosen siRNA target site in the transgene 3'UTR were synthesized with different phosphorothioate linkages, 2'-O-methyl (2'-OMe) modifications, and '2-fluoro modifications. In addition, two of these siRNAs also contained a hydrophobic moiety. In addition to these experimental siRNA molecules, three negative control scrambled sequence siRNA molecules were synthesized to match the chemical modification patterns of the designed siRNA, and sequencing alignment data confirmed these siRNA molecules did not share complementarity to any site in the mouse genome. These negative control siRNA molecules would enable detection of any adverse impact of transgene expression from intramuscular administration of an siRNA in the absence of a gene-specific silencing effect.

35 Following the AAV genome design and siRNA synthesis, seven groups of rag mice (8 mice per group) were injected in the rear leg muscle with 2×10^9 genome copies (GC) of the AAV9-CyDula construct. Thirteen days following the administration of the AAV vector, a blood sample from each mouse was obtained to establish an expression baseline of the CyDula. The next day, at two weeks after injection of the AAV construct, each mouse was injected with an siRNA molecule or a suitable control. As negative controls, one group of mice was injected with phosphate buffered saline (PBS)

and another group did not receive any injection (untreated control). Two weeks after the siRNA administration, and every subsequent two weeks, blood samples were obtained from the mice to measure potential changes in serum levels of CyDula expression via ELISA (FIG. 2).

Untreated mice, as well as mice administered PBS or the negative control scrambled siRNA sequences ("C1" and "C1-HM"), in which C1 is an unmodified scrambled siRNA and C1-HM is a scrambled siRNA that is modified to contain a hydrophobic moiety. All three groups showed no change in CyDula protein expression over a 3-month period following PBS or siRNA administration (FIGS. 3A-B). These results confirm that siRNA injection of a non-hybridizing nucleotide does not impact expression of an intramuscularly administered AAV-transgene.

Mice treated with 200 µg of a targeting siRNA T1 showed no significant changes in CyDula protein expression over time, similar to the untreated and PBS treated controls. Conversely, mice treated with 200 µg of an equivalent targeted siRNA that contained a hydrophobic moiety modification on the sense strand (T1-HM) showed significant and long-lasting reduction of CyDula protein expression. Fourteen days following T1-HM administration, CyDula protein expression was reduced by about 65% relative to the baseline levels obtained prior to siRNA administration (FIG. 4). These results indicate that a cholesterol modification significantly enhances the cellular uptake of an siRNA molecule following intramuscular administration.

Next, the durability of reduced transgene expression from intramuscular administration of cholesterol-modified siRNA molecules were evaluated. Approximately three months after receiving 200 µg of the T1-HM hydrophobic moiety-modified targeting siRNA, mice only recovered about 40% of the maximally lost expression (FIG. 5). Moreover, administration of 50 µg of a similar siRNA yielded a lower magnitude of reduced CyDula protein expression as compared to the reduction achieved with 200 µg; however, similar to the higher dose, this reduction was similarly long lasting, with mice recovering about 50% of the maximally lost expression by day 122 of the study (FIG. 6). These data collectively show that administration of a hydrophobic moiety-modified siRNA that target and hybridize a region specific to a transgene results in a surprisingly long lasting and durable reduction in transgene expression.

Example 2: Response of IM-AAV delivered serum reporter expression to administration of various doses of siRNA targeting the 3'UTR of the AAV transgene

This example describes the design and intramuscular administration of siRNA molecules for reducing expression of a transgene of interest following administration of a transgene with a designed AAV vector. A model AAV genome in an AAV9 serotype was designed to have multiple potential siRNA target sites in the 3'UTR of a transgene (FIG. 1), in which the potential siRNA target sites did not have any sequence identity to a region in a host genome (e.g., a mouse genome; e.g., a human genome). The transgene (e.g., the therapeutic transgene) was cyno dulaglutide (CyDula), a GLP-1 agonist protein that resembles dulaglutide but is modified to reduce immunogenicity in cynomolgus macaques.

An experimental siRNA (siRNA-1) was synthesized to target a sequence in the 3'UTR of the AAV transgene. The experimental siRNA contained a hydrophobic moiety modification on the 3' end to enhance muscle uptake, as well as 2'-O-Me and 2'-fluoro modifications. In addition to the

experimental siRNA molecule, a negative control scrambled sequence siRNA molecule ('non-targeting control oligo') was synthesized to match the chemical modification patterns of the designed siRNA. Sequencing alignment data confirmed the siRNA molecules did not share complementarity to any site in the mouse genome. The negative control siRNA molecule would enable detection of any adverse impact of transgene expression from intramuscular administration of an siRNA in the absence of a gene-specific silencing effect.

Following the AAV genome design and siRNA synthesis, 13 groups of rag mice (8 mice per group) were injected in the rear leg muscle with 2×10^9 genome copies (GC) of the AAV9-CyDula construct. Thirteen days following the administration of the AAV vector, a blood sample from each mouse was obtained to establish an expression baseline of the CyDula. The next day, at two weeks after injection of the AAV construct, each mouse was injected with an siRNA molecule (400 µg, 200 µg, 100 µg, 50 µg, 25 µg, 12 µg, or 6 µg per mouse in different groups) or the non-targeting control oligo (400 µg, 200 µg, 100 µg, 50 µg, per mouse in different groups) at varying doses to establish the relationship between siRNA dose and serum reporter transgene expression reduction. As negative controls, one group of mice was injected with PBS. Two weeks after the siRNA administration, blood samples were obtained from the mice to measure potential changes in serum levels of CyDula expression via ELISA.

We compared the group average fraction of CyDula (GLP-1-Fc) remaining in the serum at d28 (2 weeks after siRNA treatment) as compared to the d13 expression level, prior to the siRNA, with a scrambled siRNA as a negative control (FIG. 7). PBS treated group showed no significant drop in CyDula serum expression. Mice treated with at least 50 µg of siRNA-1 showed reductions in serum CyDula in a dose-sensitive manner, with the highest dose of 400 µg/mouse achieving >60% reporter expression reduction. In contrast, the non-targeting siRNA did not lead to reduced reporter expression. These data demonstrate that administration of a cholesterol-modified siRNA that target and hybridize a region specific to a transgene results in the reduction of transgene expression to the serum in a dose-sensitive manner.

Example 3: Extent of transgene expression reduction in muscle following the administration of various doses of siRNA targeting the 3'UTR of the AAV transgene

The mouse model for IM-AAV differs from larger animal models in that a significant portion of the injected AAV vector spills over to the liver in the mouse model, but much less so in larger animals like non-human primates, and, presumably, humans. This confounds efforts to understand the extent of transgene expression knockdown in the muscle using the mouse model, since the muscle-injected siRNAs may reach muscle far more efficiently than liver. Liver expression may therefore contribute a background level of serum reporter protein that obscures the true extent of muscle transgene expression loss. Since muscle transgene expression loss is the critical parameter in large animal (and human) systems, we next undertook an experiment designed to specifically measure muscle transgene knockdown.

Two experimental siRNAs were synthesized (siRNA-1 and siRNA-2). siRNA-1 and siRNA-2 target the same sequence within the 3'UTR of the AAV-CyDula genome (FIG. 1). All experimental

siRNAs contained a hydrophobic moiety modification to the 3' end to enhance muscle uptake, as well as 2'-O-Me and 2'-fluoro modifications. siRNA-1 and siRNA-2 differ slightly in design, including differences in the patterning of 2'-O-Me and 2'-fluoro modifications.

Thirteen groups of rag mice (8 mice per group) were injected in the rear leg muscle with 2 x 10⁹ genome copies (GC) of the AAV9-CyDula construct. Thirteen days following the administration of the AAV vector, a blood sample from each mouse was obtained to establish an expression baseline of the CyDula. The next day, at two weeks after injection of the AAV construct, each mouse was injected with a specific dose of one of the siRNA molecules. As a negative control, one group of mice was injected with PBS. Two weeks after the siRNA administration, blood samples were obtained from the mice to measure potential changes in serum levels of CyDula expression via ELISA, and the mice were sacrificed with the injected muscle tissue and a sample of liver obtained for transgene mRNA analysis.

Administration of siRNA-1 and siRNA-2 resulted in decreased serum levels of CyDula relative to a PBS-administered control at the 2-week time point following post-IM-siRNA delivery (FIG. 8). As expected, no reduction in serum CyDula protein levels were observed for PBS. siRNA-1 achieved significant reduction in serum CyDula in a dose-sensitive manner, as observed previously. siRNA-2 achieved a slightly higher level of CyDula reduction (nearly 80% at the 200 µg dose), indicating that specific siRNA design parameters can result in different initial efficacies of siRNAs targeting the same vector sequence.

Administration of siRNA-1 or siRNA-2 also resulted in decreased transgene mRNA levels in muscle tissue relative to a PBS-administered control, as measured by qPCR using a transgene-specific primer and probe set (FIG. 9). These data confirm the higher potency of siRNA-2 relative to siRNA-1 that was observed in serum data (FIG. 8). When measured at the level of muscle transgene expression (transgene muscle mRNA), siRNA-1 and siRNA-2 show more extensive knockdown than serum protein measurements would suggest (~92% muscle mRNA knockdown for siRNA-2 at the 200 µg dose vs 80% reduction in serum protein expression). This may be the result of differential siRNA knockdown efficiencies in the separate pools of transduced muscle and liver cells that likely contribute to the serum expression of the transgene protein. Nonetheless, these results confirm excellent local knockdown in muscle of up to 92%, a level that would be clinically significant for a temporary pause in gene therapy, as this would, in many cases, take the transgene out of a therapeutic window of expression.

To further explore potency for the experimental siRNA-1, remaining groups in the study received a descending dose series of the siRNA from a maximum dose of 400 µg/mouse. Comparing serum CyDula expression 2 weeks post-siRNA to pre-siRNA serum CyDula levels, we observe the expected dose-response for siRNA-1, here fitting in a simple three-parameter inhibition model to an IC₅₀ of 39 µg with a 30% maximum knockdown (FIG. 10). As discussed above, liver contributions may mask the true extent of local knockdown in the muscle in the mouse model. Therefore, we evaluated the same cohort of siRNA-1-dosed groups for transgene mRNA levels in the injected muscle using qPCR and transgene specific primer and probes. Figure 11 shows the dose-response curve for siRNA-1 in injected-muscle transgene mRNA levels (relative to PBS control). In muscle

mRNA terms, the potency of siRNA-1 is slightly higher (28 µg) with a slightly lower maximum inhibition level (<10% residual expression), consistent with the higher activity of locally IM-delivered siRNAs in the injected muscle tissue—a critical parameter for larger animal studies.

5 **Example 4: Administration of an inhibitory nucleic acid successfully pauses expression of a heterologously expressed protein**

This example illustrates that an intramuscularly administered inhibitory nucleic acid can decrease the expression of a transgene of interest for a subject in which pausing the expression presents a clinical benefit.

10 A female subject with an inherited bleeding disorder has previously undergone gene therapy to increase the expression of a circulating blood clotting protein to reduce the risk of hemorrhage. The gene therapy included administration of a recombinant AAV vector with the transgene of interest. After administration of the AAV that comprises the transgene of interest, the relative protein expression levels detected in a blood sample from the subject are determined to be in a normal range
15 (e.g., an expression level within an accepted range). After administration of the transgene (e.g., one or more days, one or more weeks, one or more months, or one or more years after administration of the transgene), the subject is determined to be pregnant.

A skilled practitioner (e.g., a clinician) determines that the expression level of the circulating protein has increased relative to the expression levels prior to pregnancy and poses a potential risk
20 for an adverse event (e.g., a blood clot and/or preeclampsia). To reduce the risk of developing one or more of adverse events, the subject undergoes treatment with an inhibitory nucleic acid (e.g., an siRNA; e.g., an siRNA modified to contain a hydrophobic moiety) to reduce the expression of the transgene that encodes the circulating protein. The inhibitory nucleic acid is complementary to a region of the transcript transcribed by the transgene (e.g., a region upstream of the start codon, a
25 region downstream of the stop codon, a region in the 5'UTR, a region in the 3'UTR, or another suitable region described herein) but is not complementary to a region in the subject's genome (e.g., a DNA sequence or an RNA sequence; e.g., an endogenous mRNA sequence that encodes the protein of interest). Thus, administration of the inhibitory nucleic acid will reduce the protein that is expressed by the transgene but will not impact endogenous protein expression of the blood clotting
30 protein. The subject is intramuscularly administered an effective amount of the inhibitory nucleic acid that is sufficient to reduce expression of the transgene for the remainder of the subject's pregnancy (e.g., about 9 months, about 8 months, about 7 months, about 6 months, about 5 months, about 4 months, about 3 months, about 2 months, or about 1 month). Measurements of the protein expression level in a blood sample obtained from the subject determine that the protein expression levels are
35 within a normal range.

Example 5: Temporally modulating expression of a transgene for effective treatment of a disease or condition

This example illustrates a method of temporally modulating the expression of a gene such as
40 a transgene that encodes a polypeptide or a protein of interest or a fragment thereof (e.g., a

therapeutic polypeptide or a therapeutic protein or fragment thereof) by administering to a subject a polynucleotide that comprises the transgene to increase expression of the transgene and then administering to the subject an inhibitory nucleic acid to decrease the expression of the transgene.

5 A subject (e.g., a human subject) has or is at risk of having a disease or a condition (e.g., a disease or a condition listed in Table 1) is treated with a form of gene therapy that comprises intramuscularly administering to the subject an effective amount pharmaceutical composition comprising a polynucleotide that comprises (i) a transgene that encodes a therapeutic polypeptide or a therapeutic protein or a fragment thereof and (ii) a region that has complementarity to one or more inhibitory nucleic acids (e.g., an inhibitory RNA molecule). The pharmaceutical composition may 10 further comprise a delivery vehicle for effective delivery of the polynucleotide to the subject, in which the delivery vehicle is a vector such as a viral genome (e.g., an AAV vector (e.g., a recombinant AAV vector)), a liposome, or a microvesicle, among other suitable forms of delivery described herein. The pharmaceutical composition may further comprise components of a genetic engineering system (e.g., CRISPR or a transposon system) for delivering the polynucleotide to the subject.

15 After intramuscular administration of the pharmaceutical composition comprising the polynucleotide (e.g., more than one day after, more than one week after, more than one month after, more than one year after, and/or more than five years after) a biological sample (e.g., a blood sample or a sample from a biopsy) is obtained from the subject and confirms that measured expression levels of the transgene (e.g., protein expression levels and mRNA transcript expression levels) are 20 comparable to expression levels detected in a suitable control (e.g., a biological sample from a healthy subject (e.g., a subject that does not have or is not at risk of having the disease or condition of the subject), and/or an accepted range of expression levels).

After administering the pharmaceutical composition comprising the polynucleotide (e.g., more than one day after, more than one week after, more than one month after, more than one year after, 25 and/or more than five years after), and optionally determining an increase in expression of the transgene, the subject is determined to have or at risk of having an indication for which subsequently decreasing the expression of the transgene is desirable. The subject is further intramuscularly administered an effective amount of a pharmaceutical composition comprising an inhibitory nucleic acid (e.g., an inhibitory RNA molecule; e.g., an siRNA, a dsRNA, an miRNA, an shRNA, an ASO, a 30 gapmer), in which the inhibitory nucleic acid may further comprise one or more modifications, such as one or more modifications described herein. The inhibitory nucleic acid comprises a region that is complementary (at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% complementary) to a region of the transgene (e.g., an mRNA transcript transcribed by the transgene). Administration of the pharmaceutical composition comprising an 35 inhibitory nucleic acid reduces the expression level of the transgene by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% as compared to the expression level of the transgene prior to administration of the pharmaceutical composition comprising an inhibitory nucleic acid, indicating that the treatment for 40 decreasing the transgene expression is successful.

Other Embodiments

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

- 5 While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations following, in general, the principles and including such departures from the invention that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope
- 10 of the claims.

Other embodiments are within the claims.

CLAIMS

1. A method of reducing expression or activity of a protein of interest in a subject, the method comprising:
 - (a) administering to the subject a recombinant adeno-associated viral (AAV) vector comprising (i) a transgene encoding the protein of interest and (ii) a binding site for an inhibitory RNA molecule; and subsequently
 - (b) administering to the subject an inhibitory RNA molecule, wherein the inhibitory RNA molecule has complementarity to the binding site in the AAV vector, optionally wherein the recombinant AAV is administered to the subject intramuscularly.
2. A method of reducing expression or activity of a protein of interest in a subject, the method comprising administering to the subject an inhibitory RNA molecule, wherein the subject has previously been administered a recombinant AAV vector comprising (i) a transgene encoding the protein of interest and (ii) a binding site having complementarity to the inhibitory RNA molecule, optionally wherein the recombinant AAV is administered to the subject intramuscularly.
3. A method of temporally modulating expression or activity of a protein of interest in a subject, the method comprising:
 - (a) administering to the subject a recombinant AAV vector comprising (i) a transgene encoding the protein of interest and (ii) a binding site for an inhibitory RNA molecule, wherein administration of the recombinant AAV increases the expression or activity of the protein of interest in the subject; and subsequently
 - (b) administering to the subject an inhibitory RNA molecule, wherein the inhibitory RNA molecule has complementarity to the binding site in the AAV vector, wherein administration of the inhibitory RNA molecule reduces the expression or activity of the protein of interest in the subject; optionally wherein the recombinant AAV is administered to the subject intramuscularly.
4. A method of treating a disease in a subject in need thereof, wherein the disease is one that (i) is associated with or caused by a reduction in expression or activity of a protein of interest relative to a subject that does not have the disease, or (ii) is ameliorated by increasing expression or activity of a protein of interest in the subject, the method comprising:
 - (a) administering to the subject a recombinant AAV vector comprising (i) a transgene encoding a protein of interest and (ii) a binding site for an inhibitory RNA molecule; and subsequently
 - (b) administering to the subject an inhibitory RNA molecule, wherein the inhibitory RNA molecule has complementarity to the binding site in the AAV vector, optionally wherein the recombinant AAV is administered to the subject intramuscularly.
5. A method of treating a disease in a subject in need thereof, wherein the disease is one that (i) is associated with or caused by a reduction in expression or activity of a protein of interest relative to a subject that does not have the disease, or (ii) is ameliorated by increasing expression or activity of a

protein of interest in the subject, the method comprising administering to the subject an inhibitory RNA molecule, wherein the subject has previously been administered a recombinant AAV vector comprising (i) a transgene encoding the protein of interest and (ii) a binding site having complementarity to the inhibitory RNA molecule, optionally wherein the recombinant AAV is administered to the subject intramuscularly.

6. The method of any one of claims 1-5, wherein the recombinant AAV is administered to the subject between one and ten times, optionally wherein the recombinant AAV is administered to the subject one, two, three, four, five, six, seven, eight, nine, or ten times.
7. The method of claim 6, wherein the recombinant AAV is administered to the subject between one and five times, optionally wherein the recombinant AAV is administered to the subject one, two, three, four, or five times.
8. The method of claim 7, wherein the recombinant AAV is administered to the subject between one and three times, optionally wherein the recombinant AAV is administered to the subject one, two, or three times.
9. The method of claim 8, wherein the recombinant AAV is administered to the subject one or two times.
10. The method of claim 9, wherein the recombinant AAV is administered to the subject once.
11. The method of any one of claims 1-10, wherein the subject has or is at risk of developing a disease listed in Table 1.
12. The method of any one of claims 1-11, wherein the transgene is a gene listed in Table 1.
13. The method of any one of claims 1-12, wherein the expression of the protein of interest is increased following administration of the recombinant AAV.
14. The method of claim 13, wherein the expression of the protein of interest is sustained for at least five days following administration of the recombinant AAV.
15. The method of claim 14, wherein the expression of the protein of interest is sustained for at least thirty days following administration of the recombinant AAV.
16. The method of claim 15, wherein the expression of the protein of interest is sustained for at least sixty days following administration of the recombinant AAV.

17. The method of claim 16, wherein the expression of the protein of interest is sustained for at least ninety days following administration of the recombinant AAV.
18. The method of claim 17, wherein the expression of the protein of interest is sustained for at least 120 days following administration of the recombinant AAV.
19. The method of claim 18, wherein the expression of the protein of interest is sustained for at least one year following administration of the recombinant AAV.
20. The method of claim 19, wherein the expression of the protein of interest is sustained between one to five years following administration of the recombinant AAV.
21. The method of claim 20, wherein the expression of the protein of interest is sustained for at least 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, or more than 10 years following administration of the recombinant AAV.
22. The method of any one of claims 1-21, wherein the protein of interest is expressed at a therapeutic or sub-therapeutic level following administration of the recombinant AAV.
23. The method of any one of claims 1-22, wherein the inhibitory RNA molecule is selected from a small interfering RNA (siRNA), an antisense oligonucleotide (ASO), a double-stranded RNA (dsRNA), a short hairpin RNA (shRNA), a microRNA (miRNA), or a gapmer.
24. The method of claim 23, wherein the inhibitory RNA molecule is an siRNA.
25. The method of claim 23 or 24, wherein the inhibitory RNA molecule further comprises a modification selected from a chemically modified sugar, chemically modified nucleobase, and a chemically modified internucleoside linkage.
26. The method of any one of claims 23-25, wherein the inhibitory RNA molecule further comprises a hydrophobic moiety.
27. The method of claim 26, wherein the hydrophobic moiety is a cholesterol.
28. The method of any one of claims 23-27, wherein the inhibitory RNA molecule is formulated in a delivery vehicle.
29. The method of claim 28, wherein the delivery vehicle is a lipid-based carrier, a liposome, or a lipid nanoparticle.

30. The method of any one of claims 1-29, wherein the inhibitory RNA molecule lacks sufficient complementarity to hybridize to an endogenous RNA sequence that naturally occurs in a cell of the subject.
31. The method of claim 30, wherein the inhibitory RNA molecule has complementarity to an mRNA transcript, or a portion thereof, that is transcribed from the recombinant AAV vector and that encodes the protein of interest.
32. The method of claim 31, wherein the inhibitory RNA molecule has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% complementarity to a region within the mRNA transcript.
33. The method of claim 32, wherein the inhibitory RNA molecule has 100% complementarity to the region within the mRNA transcript.
34. The method of any one of claims 31-33, wherein the inhibitory RNA molecule contains a sequence that has complementarity to at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides within the mRNA transcript.
35. The method of any one of claims 1-34, wherein the inhibitory RNA molecule binding site is present within the 3' untranslated region (UTR) of the transgene, the 5' UTR of the transgene, a region upstream of the start codon in the transgene, a region downstream of the stop codon in the transgene, or a region in the open reading frame of the transgene.
36. The method of any one of claims 1-35, wherein the inhibitory RNA molecule is intramuscularly administered to the subject.
37. The method of any one of claims 1-36, wherein administration of the inhibitory RNA molecule reduces expression of the protein of interest by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%, as compared to expression of the protein of interest in the subject prior to administration of the inhibitory RNA molecule.
38. The method of any one of claims 1-36, wherein administration of the inhibitory RNA molecule reduces the expression of the protein of interest by about 2-fold, by about 3-fold, by about 4-fold, by about 5-fold, by about 6-fold, by about 7-fold, by about 8-fold, by about 9-fold, by about 10-fold, by about 15-fold, by about 20-fold, by about 25-fold, by about 30-fold, by about 35-fold, by about 40-fold, by about 45-fold, by about 50-fold, or greater than 50-fold, as compared to expression of the protein of interest in the subject prior to administration of the inhibitory RNA molecule.

39. The method of claim 37 or 38, wherein administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least five days.
40. The method of claim 39, wherein administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least thirty days.
41. The method of claim 40, wherein administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least sixty days.
42. The method of claim 41, wherein administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least ninety days.
43. The method of claim 42, wherein administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least 120 days.
44. The method of claim 43, wherein administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least one year.
45. The method of claim 44, wherein administration of the inhibitory RNA molecule reduces expression of the protein of interest for at least five years.
46. The method of any one of claims 1-45, wherein the AAV vector is a serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, rh10, or rh74 AAV vector, or wherein the AAV vector is encapsulated by one or more synthetic capsid proteins.
47. The method of any one of claims 1-46, wherein the inhibitory RNA molecule is administered to the subject in advance of the subject receiving a therapeutic intervention that is contraindicated with the protein of interest.
48. The method of claim 47, wherein the inhibitory RNA molecule is administered to the subject from one day to 12 months prior to the subject receiving a therapeutic intervention that is contraindicated with the protein of interest.
49. The method of claim 48, wherein the inhibitory RNA molecule is administered to the subject one week, one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, 11 months, or 12 months prior to receiving a therapeutic intervention that is contraindicated with the protein of interest.
50. The method of any one of claims 1-49, wherein the subject is a human.