

THERAPY FOR ALPHA-1 ANTITRYPSIN DEFICIENCY**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 63/512,773, filed July 10, 2023.

BACKGROUND OF THE DISCLOSURE

[0002] Alpha-1 Antitrypsin Deficiency (AATD) is a genetic disorder that affects a significant number of individuals, particularly those with European ancestry. It is estimated to impact approximately 1 in 1,500 to 3,500 individuals within this population, although it can also occur less commonly in other ethnic groups.

[0003] This genetic disorder results from mutations in the SERPINA1 gene that encodes the alpha-1 antitrypsin (AAT) protein, which is synthesized in the liver. One such mutation involves a single nucleotide change that results in the substitution of a lysine amino acid for a glutamic acid at position 342 in the AAT protein sequence, leading to the production of the AAT(Z) variant. Another such mutation results in the AAT(S) variant, which involves a mutation wherein methionine at position 264 of the AAT protein is replaced by valine. The AAT(I) variant results when isoleucine is substituted for methionine at position 50 of the AAT protein. Another variant, AAT(Siiyama), results when lysine is substituted for glutamate at position 288 of the AAT protein. Yet another variant, the AAT(Mmalton), results when a four amino acid segment of the AAT protein is deleted. In each of these cases, the mutations result in a form of AAT that is misfolded in comparison with normal AAT (encoded by variant AAT(M), exemplary sequence of which can be found at SEQ ID NO: 600), causing it to be inefficiently secreted from the liver. This leads to the aggregation of mutant AAT in the liver and reduced amounts of normal, functional AAT in the bloodstream.

[0004] AAT functions as a protease inhibitor and plays a crucial role in protecting lung tissue from damage caused by enzymes released by immune cells, particularly neutrophil elastase. Without sufficient levels of functional AAT, individuals with AATD are at an increased risk of developing early-onset emphysema, chronic obstructive pulmonary disease (COPD), and other respiratory conditions. About 75% of AATD patients develop emphysema and about 10% of AATD children and adults develop liver cirrhosis and require liver transplant. Symptoms of AATD include chronic cough, shortness of breath and wheezing. Meanwhile, the abnormal accumulation of mutant AAT in the liver

can lead to liver disease, including inflammation, fibrosis, and, in severe cases, cirrhosis and hepatocellular carcinoma.

[0005] Currently, the FDA-approved treatment for AATD involves regular infusions of plasma-derived AAT protein throughout an affected individual's lifetime in order to maintain adequate levels of the protein in the patient's blood. This therapy aims to restore the protective function of AAT in the lungs and reduce the progression of respiratory symptoms. However, there is no approved treatment available to address the associated liver disease that can occur in individuals with AATD. Thus, there is an urgent need for effective therapies that can address both the pulmonary and hepatic manifestations of AATD.

[0006] Gene therapy using microRNAs to silence expression of the allele expressing mutant AAT while also presenting a transgene allowing for expression of normal, functional AAT presents an alternative approach for treatment of AATD.

[0007] MicroRNAs (miRNAs) are small non-coding RNA molecules that bind to mRNA molecules produced by specific genes, influencing their protein translation process or by destabilizing the mRNA transcript. Through this mechanism, miRNAs effectively silence the expression of the target gene.

[0008] Previous approaches to enhance gene silencing involve encoding multiple miRNAs within a single polycistronic genetic construct, which can be delivered to cells using vectors, as illustrated in Mueller et al., *Molecular Therapy*, 20:590–600 (2012) ("Mueller"), Figure 1. This method has demonstrated robust reduction of the target gene. However, when a construct containing repetitive precursor miRNA (pre-miRNA) structures is employed, there is a risk of alternative folding of the pre-miRNA stem-loop structures during transcription. This alternative folding may generate alternatively processed miRNAs, inadvertently causing off-target gene silencing, which poses potential safety concerns. Additionally, using a construct with repetitive pre-miRNA structures may enable recombination within the vector, resulting in the production of impure vector populations with sequence variations.

[0009] To address these risks, the present invention involves a polycistronic construct that encodes multiple distinct pre-miRNAs to reduce expression of the allele expressing mutant AAT, ensuring that the pre-miRNAs are non-complementary to one another. In certain embodiments, a minimum separation of approximately 7 nucleotides is maintained between pre-miRNA structures to promote appropriate co-transcriptional folding of the RNA. Moreover, in specific embodiments, the pre-miRNAs are designed to preserve the predicted stem-loop structure and internal loops based on

endogenous human sequences, thus minimizing the risk of RNAi-based toxicity. These constructs can incorporate pre-miRNAs that target different genes or distinct regions of the same gene.

SUMMARY OF THE DISCLOSURE

[0010] The present invention relates to a therapeutic genetic construct encoding dual pre-miRNA and normal, functional AAT where the dual miRNA are used to reduce expression of mutant variant(s) of AAT, such as AAT(Z), while introducing the AAT(M) transgene encoding normal, functional AAT.

[0011] In certain embodiments, the genetic construct of the present invention comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 592 (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 592), a codon degenerate variant of SEQ ID NO: 592, or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 592).

[0012] In certain embodiments, the dual miRNA of the present invention comprise a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 589 (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 589) or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 589.

[0013] In certain embodiments, the transgene expressing the AAT(M) variant is modified so as to evade reduction of expression by miRNAs. In one embodiment, the miRNA-resistant AAT(M) transgene comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 590 (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 590), a codon degenerate variant of SEQ ID NO: 590, or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 590.

[0014] In certain embodiments, the miRNA-resistant AAT(M) transgene is modified to have reduced levels of CG dinucleotides (also referred to as CpG). In one embodiment, the CpG-reduced miRNA-resistant transgene comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 596 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 596), a codon degenerate variant of SEQ ID NO: 596, or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 596.

[0015] In certain embodiments, the AAT(M) transgene encodes an amino acid sequence having at least about 80% sequence identity with SEQ ID NO: 600 (*e.g.*, an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 600), or a conservatively-substitute variant of SEQ ID NO: 600.

[0016] In certain embodiments, the genetic construct comprises a tissue-specific promoter. In one embodiment, the tissue-specific promoter is specific to the lungs. In another embodiment, the tissue-specific promoter is specific to the liver. In a further embodiment, the liver-specific promoter comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 587 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 587) or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 587.

[0017] In another embodiment, the tissue-specific promoter is modified to have reduced levels of CpG. In one embodiment, the CpG-reduced tissue-specific promoter is specific to the liver. In a further embodiment, the CpG-reduced liver-specific promoter comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 594 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 594) or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 594.

[0018] In certain embodiments, a vector is used to introduce the genetic construct of the present invention into a cell. In certain embodiments, the vector is an adenovirus or adenoviral vector. In preferred embodiments, the vector is an adenovirus vector derived from gorilla, referred to as a gorilla adenovirus vector, like GC44, GC45, or GC46. In one embodiment, the vector comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 593 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 593), a codon degenerate variant of SEQ ID NO: 593, or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 593.

[0019] The present invention also relates in part to a pharmaceutical composition comprising the vector described herein and a pharmaceutically-acceptable carrier.

[0020] The present invention also relates in part to a method of treating a disease or disorder in a

subject in need thereof comprising administering the genetic construct and/or vector described herein, or a composition comprising the same, to the subject. In an embodiment, the disease or disorder is a liver or lung disease associated with alpha-1 antitrypsin deficiency.

[0021] The present invention also relates in part to the use of the genetic construct and/or vector described herein, or a composition comprising the same, in the manufacture of a medicament for use in treating a disease or disorder in a subject in need thereof. In preferred embodiments, the genetic constructs described herein, or any vector comprising any genetic construct of the present invention, or a composition comprising such a nucleic acid or vector, are used to treat liver or lung disease associated with alpha-1 antitrypsin deficiency.

[0022] The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. 1A depicts the location of expression of a firefly luciferase transgene in BALB/C mice on Day 2 following intravenous administration of a 1×10^{10} PU dose of a GC44 vector encoding firefly luciferase under the control of a CMV promoter.

[0024] FIG. 1B depicts the location of expression of the firefly luciferase transgene in BALB/C mice on Day 2 following intravenous administration of a 1×10^{10} PU dose of a GC44 vector encoding firefly luciferase under the control of a liver specific promoter (LSP).

[0025] Fig. 1C depicts the location of expression of a firefly luciferase transgene in NSG-PiZ mice on Day 1 following intravenous administration of a 1×10^{11} PU dose of a vector encoding firefly luciferase under the control of a CMV promoter or a liver specific promoter (LSP).

[0026] FIG. 2A depicts total flux (p/s) in mice following administration of 1×10^9 , 1×10^{10} , or 1×10^{11} PU of a GC44 vector encoding firefly luciferase under the control of a CMV promoter or a liver specific promoter (LSP).

[0027] Fig. 2B depicts total flux (p/s) in mice following administration of 1×10^9 , 1×10^{10} , or 1×10^{11} PU of a vector encoding firefly luciferase under the control of a CMV promoter or a liver specific promoter (LSP).

[0028] FIG. 3A depicts the presence of AAT(Z) in the liver of an untreated NSG-PiZ mouse by PAS-

D staining.

[0029] FIG. 3B depicts the presence of AAT(Z) in the liver of an untreated NSG-PiZ mouse 28 days following administration of FFB control buffer.

[0030] FIG. 4A depicts the presence of AAT(Z) in the liver of an NSG-PiZ mouse that has been administered a GC44 vector encoding miRNA targeting AAT(Z) and the AAT(M) transgene.

[0031] FIG. 4B depicts the presence of AAT(Z) in the liver of an untreated NSG-PiZ mouse 28 days following administration of 1×10^{11} PU of a GC44 vector encoding dual miRNA targeting AAT(Z) and the AAT(M) transgene.

[0032] Fig. 5 depicts the number of copies of adenovirus per cell in various mouse organs. Groups are the same as in Fig. 16A-16D.

[0033] Fig. 6 depicts a table showing the fold change in AAT transgene RNA expression over Group 1 reference sample relative to other tissues. Groups are the same as in Fig. 16A-16D.

[0034] FIG. 7A depicts the levels of human AAT(M) (hAAT(M)) in C57BL/6 mice following treatment with a GC44 vector encoding dual miRNA targeting AAT(Z) and the AAT(M) transgene.

[0035] FIG. 7B depicts the levels of human AAT(M) (hAAT(M)) in NSG mice following treatment with a GC44 vector encoding dual miRNA targeting AAT(Z) and the AAT(M) transgene.

[0036] FIG. 8A depicts the level of human AAT(M) secreted into media of HepG2 liver cell line cultures transfected with: (A) plasmid DNA encoding a liver specific promoter, 5'UTR, dual miRNA targeting AAT(Z), the AAT(M) transgene, and a 3'UTR (indicated as 5924); (B) plasmid DNA encoding a liver specific promoter, 5'UTR, dual miRNA targeting AAT(Z), a CpG-reduced version of the AAT(M) transgene, and a 3'UTR (indicated as 6568); (C) plasmid DNA encoding a CpG-reduced version of a liver specific promoter, 5'UTR, dual miRNA targeting AAT(Z), a CpG-reduced version of the AAT(M) transgene, and a 3'UTR (indicated as 6569); (D) plasmid DNA encoding a liver specific promoter, a CpG-reduced 5'UTR, dual miRNA targeting AAT(Z), a CpG-reduced version of the AAT(M) transgene, and a 3'UTR (indicated as 6570); and (E) plasmid DNA encoding a CpG-reduced version of a liver specific promoter, a CpG-reduced 5'UTR, dual miRNA targeting AAT(Z), a CpG-reduced version of the AAT(M) transgene, and a 3'UTR (indicated as 6571).

[0037] Fig. 8B depicts the relative body weight of mice administered the RA-1276 or RA-1330 vector construct compared to the control (final formulation buffer (FFB)).

[0038] Fig. 9 depicts the concentration (μM) of human AAT (hAAT) in serum of mice previously administered the RA-1276 or RA-1330 vector construct compared to the control (FFB).

[0039] Fig. 10 depicts the relative body weight by percentage of mice administered various formulations. Body weight is relative to the beginning of the experiment. Groups are the same as in Fig. 12A–12D.

[0040] FIG. 11 depicts images of PAS-D stained liver cells from NSG-PiZ mice 28 days following administration of a GC44 vector encoding dual miRNA targeting AAT(Z) and the AAT(M) transgene at 1×10^{11} PU, 5×10^{10} , 1×10^{10} , 5×10^9 , and 1×10^9 PU, a control NSG-PiZ mouse not administered such vector, and a control NSG mouse (not expressing AAT(Z)).

[0041] Fig. 12A–12D illustrate the efficacy of GC44 and GC45 vectors in mice. Group 1 is NSG mice administered a control formulation (FFB). Group 2 is NSG-PiZ mice administered a control formulation (FFB). Group 3 is NSG-PiZ mice administered RA-1275 vector (a GC44 vector with a liver-specific promoter driving expression of a dual miRNA construct and AAT(M) expression). Group 4 is NSG-PiZ mice administered RA-1330 vector (a GC44 vector with a CpG reduction and a liver-specific promoter driving expression of a dual miRNA construct and AAT(M) expression). Group 5 is NSG-PiZ mice administered a RA-1331 (a GC45 vector with a CpG reduction and a liver-specific promoter driving expression of a dual miRNA construct and AAT(M) expression). Fig. 12A depicts representative images of liver tissue stained with PAS-D staining to show liver globules. Fig. 12B depicts the relative expression of AAT(Z) allele transcript of SERPINA1 in NSG-PiZ mice at 28 days post-administration. Fig. 12C depicts the number of copies of AAT(M) allele in mouse liver. Fig. 12D depicts the number of copies of adenovirus per cell in mouse liver.

[0042] Fig. 13 is a schematic diagram of a study assessing expression behavior of vectors comprising miRNA dual designs targeting AAT(Z), a liver-specific promoter, miRNA-resistant AAT(M) transgene, 5'UTR, and 3'UTR (Groups 4–6, NSG-PiZ mice).

[0043] Fig. 14A is a representative image of PAS-D staining in liver tissues from m NSG-PiZ mice 28 days following administration of each vector design. Fig. 14B is a graph of the % PAS-D staining in the liver tissues of Fig. 14A at day 28.

[0044] Fig. 15 depicts the liver-specific detection of adenovector DNA relative to lowest detected sample and relative to detection in other tissues in NSG-PiZ mice, following administration of vectors comprising miRNA dual designs targeting AAT(Z), a liver-specific promoter, miRNA-resistant

AAT(M) transgene, 5'UTR, and 3'UTR (Groups 4-6) at days 14 and 28 post-collection. The day 14 value for Group 3 pancreas expression is crossed out because it appears artificially high. The detection of DNA was below the lower limit of quantitation.

[0045] Fig. 16A depicts a dot plot showing the liver-specific AAT(M) transgene expression at day 28 following treatment with vectors comprising miRNA dual designs targeting AAT(Z), a liver-specific promoter, miRNA-resistant AAT(M) transgene, 5'UTR, and 3'UTR (Groups 4-6, NSG-PiZ mice). FIG. 16B depicts a table showing the fold change in AAT transgene RNA expression relative to the Group 1 reference sample across different tissues.

[0046] Figs. 17A&B depict miRNA expression in the liver (NSG-PiZ mice) at day 28 (dot plot – Fig. 17A) and on average throughout the 28 days (table – FIG. 17B) relative to miRNA expression in other tissues following administration of vectors comprising miRNA dual designs targeting AAT(Z), a liver-specific promoter, miRNA-resistant AAT(M) transgene, 5'UTR, and 3'UTR (Groups 4-6).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0047] The following description and examples illustrate embodiments of the present disclosure in detail. It is to be understood that the present disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are variations and modifications of the present disclosure, which are encompassed within the scope of the present invention.

[0048] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0049] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0050] Although various features of the disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment.

I. Definitions

[0051] The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.*, to any commonly owned patent or application. The terminology used herein is for the purpose of describing particular

embodiments only, and is not intended to be limiting.

[0052] In this application, the use of the singular includes the plural unless specifically stated otherwise. As used in the specification, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

[0053] In this application, the use of “or” means “and/or” unless stated otherwise. The terms “and/or” and “any combination thereof” and their grammatical equivalents can be used interchangeably. These terms can convey that any combination is specifically contemplated. Solely for illustrative purposes, the following phrases “A, B, and/or C” or “A, B, C, or any combination thereof” can mean “A individually; B individually; C individually; A and B; B and C; A and C; and A, B, and C.” The term “or” can be used conjunctively or disjunctively, unless the context specifically refers to a disjunctive use.

[0054] Furthermore, use of the term “including” as well as other forms, such as “include,” “includes,” and “included,” is not limiting.

[0055] Reference in the specification to “some embodiments,” “an embodiment,” “one embodiment” or “other embodiments” means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

[0056] As used in this specification and the claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[0057] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. In another example,

the amount “about 10” includes 10 and any amounts from 9 to 11. In yet another example, the term “about” in relation to a reference numerical value can also include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value. Alternatively, particularly with respect to biological systems or processes, the term “about” can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0058] A “therapeutically-effective amount” or “therapeutically-effective dose” refers to an amount or dose effective, for periods of time necessary, to achieve a desired therapeutic result. The amount can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the inventive nucleic acid sequences to elicit a desired response in the individual.

[0059] “Polynucleotide” or “oligonucleotide” refers to a polymeric form of nucleotides or nucleic acids of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double and single stranded deoxyribonucleic acid (DNA), triplex DNA, as well as double and single stranded ribonucleic acid (RNA). It also includes modified, for example, by methylation and/or by capping, and unmodified forms of the polynucleotide. The term is also meant to include molecules that include non-naturally occurring or synthetic nucleotides as well as nucleotide analogs.

[0060] Unless otherwise stated, nucleic acid sequences in the text of this specification are given, when read from left to right, in the 5' to 3' direction.

[0061] The terms “transfection,” “transformation,” “nucleofection,” or “transduction” refer to the introduction of one or more exogenous polynucleotides into a host cell or organism by using physical, chemical, and/or electrical methods. The nucleic acid sequences and vectors disclosed herein can be introduced into a cell or organism by any such methods, including, for example, by electroporation, calcium phosphate co-precipitation, strontium phosphate DNA co-precipitation, liposome mediated-transfection, DEAE dextran mediated-transfection, polycationic mediated-transfection, tungsten particle-facilitated microparticle bombardment, viral, and/or non-viral mediated transfection. In some cases, the method of introducing nucleic acids into the cell or organism involves the use of viral, retroviral, lentiviral, or transposon, or transposable element-mediated (e.g., *Sleeping Beauty*) vectors.

[0062] “Polypeptide,” “peptide,” and their grammatical equivalents refer to a polymer of amino acid residues. The polypeptide can optionally include glycosylation or other modifications typical for a

given protein in a given cellular environment. Polypeptides and proteins disclosed herein (including functional fragments and functional variants thereof) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α -amino n-decanoic acid, homoserine, S-acetylaminoethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β -phenylserine β -hydroxyphenylalanine, phenylglycine, α -naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α -aminocyclopentane carboxylic acid, α -aminocyclohexane carboxylic acid, α -aminocycloheptane carboxylic acid, α -(2-amino-2-norbornane)-carboxylic acid, α,γ -diaminobutyric acid, α,β -diaminopropionic acid, homophenylalanine, and α -tert-butylglycine. The present disclosure further contemplates that expression of polypeptides or proteins described herein in an engineered cell can be associated with post-translational modifications of one or more amino acids of the polypeptide or protein. Non-limiting examples of post-translational modifications include phosphorylation, acylation including acetylation and formylation, glycosylation (including N-linked and O-linked), amidation, hydroxylation, alkylation including methylation and ethylation, ubiquitylation, addition of pyrrolidone carboxylic acid, formation of disulfide bridges, sulfation, myristoylation, palmitoylation, isoprenylation, farnesylation, geranylation, glypiation, lipoylation and iodination.

[0063] The term “conservative amino acid substitution” or “conservative mutation” refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., *Principles of Protein Structure*, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids can be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., *supra*). Examples of conservative mutations include amino acid substitutions of amino acids within the sub-groups below, for example, lysine for arginine and vice versa such that a positive charge can be maintained; glutamic acid for aspartic acid and vice versa such that a negative charge can be maintained; serine for threonine such that a free –OH can be maintained; and glutamine for asparagine such that a free –NH₂ can be maintained.

Exemplary conservative amino acid substitutions are shown in the following chart:

<u>Type of Amino Acid</u>	<u>Substitutable Amino Acids</u>
Hydrophilic	Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser, Thr
Sulphydryl	Cys
Aliphatic	Val, Ile, Leu, Met
Basic	Lys, Arg, His
Aromatic	Phe, Tyr, Trp

An amino acid sequence that differs from a reference amino acid sequence by only conservative amino acid substitutions will be referred to herein as a “conservatively-substituted variant” of the reference sequence.

[0064] In some embodiments, the functional variants can comprise the amino acid sequence of the reference protein with at least one non-conservative amino acid substitution. The term “non-conservative mutations” involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with, or inhibit the biological activity of, the functional variant. The non-conservative amino acid substitution can enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the homologous parent protein. Amino acid substitutability is discussed in more detail, for example, in L. Y. Yampolsky and A. Stoltzfus, “The Exchangeability of Amino acids in Proteins,” *Genetics* 2005 Aug.; 170(4):1459-1472.

[0065] The terms “identical” and “sequence identity,” when used in the context of two nucleic acid sequences or two amino acid sequences, refer to the nucleotides or residues in the two sequences, which are the same when aligned for maximum correspondence over a specified comparison window. A “comparison window” refers to a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence can be compared to a reference sequence of the same number of contiguous positions after the two sequences are aligned optimally. Methods of alignment of sequences for comparison are well-known in the art. Optimal

alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.*, 2:482 (1981); by the alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.*, 48:443 (1970); by the search for similarity method of Pearson and Lipman, *Proc. Nat. Acad. Sci U.S.A.*, 85:2444 (1988); by computerized implementations of these algorithms (including, but not limited to CLUSTAL in the PC/Gene program by Intelligentics, Mountain View Calif., GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis., U.S.A.); the CLUSTAL program is well described by Higgins and Sharp, *Gene*, 73:237-244 (1988) and Higgins and Sharp, *CABIOS*, 5:151-153 (1989); Corpet et al., *Nucleic Acids Res.*, 16:10881-10890 (1988); Huang et al., *Computer Applications in the Biosciences*, 8:155-165 (1992); and Pearson et al., *Methods in Molecular Biology*, 24:307-331 (1994). Alignment is also often performed by inspection and manual alignment. In one class of embodiments, the polypeptides herein are at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 99% or about 100% identical to a reference polypeptide (*i.e.*, the full length thereof), or a fragment thereof, *e.g.*, as measured by BLASTP (or CLUSTAL, or any other available alignment software) using default parameters. Similarly, nucleic acids can also be described with reference to a starting nucleic acid, *e.g.*, they can be 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to a reference nucleic acid (*i.e.*, the full length thereof) or a fragment thereof, *e.g.*, as measured by BLASTN (or CLUSTAL, or any other available alignment software) using default parameters. When one molecule is said to have certain percentage of sequence identity with a larger molecule, it means that when the two molecules are optimally aligned, the percentage of residues in the smaller molecule finds a match residue in the larger molecule in accordance with the order by which the two molecules are optimally aligned.

[0066] For purposes of this specification and the claims, it is understood that the phrase “having at least about 50% sequence identity with” a reference sequence, or referencing any range therein (*e.g.*, “at least about 80% sequence identity with”) encompasses the reference sequence itself. Thus, for example, a claim reciting “a nucleic acid having at least about 80% sequence identity with SEQ ID NO: 0” encompasses SEQ ID NO: 0 itself.

[0067] The term “substantially identical” and its grammatical equivalents as applied to nucleic acid or amino acid sequences mean that a nucleic acid or amino acid sequence comprises a sequence that has at least 95% sequence identity with a reference sequence using the programs described above, *e.g.*, BLAST, using standard parameters.

[0068] “Homology” is generally inferred from sequence identity between two or more nucleic acids or proteins (or sequences thereof). The precise percentage of identity between sequences that is useful in establishing homology varies with the nucleic acid and protein at issue, but as little as 25% sequence identity is routinely used to establish homology. Higher levels of sequence identity, *e.g.*, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more can also be used to establish homology. Methods for determining sequence identity percentages (*e.g.*, BLASTP and BLASTN using default parameters) are described herein and are generally available. Nucleic acids and/or nucleic acid sequences are “homologous” when they are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. Proteins and/or protein sequences are “homologous” when their encoding DNAs are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. The homologous molecules can be termed “homologs.” For example, any naturally occurring proteins can be modified by any available mutagenesis method. When expressed, this mutagenized nucleic acid encodes a polypeptide that is homologous to the protein encoded by the original nucleic acid.

[0069] Also contemplated and included herein are nucleic acid molecules that hybridize to the disclosed sequences. Hybridization conditions may be mild, moderate, or stringent, as is warranted.

[0070] Appropriate stringency conditions which promote DNA hybridization, for example, 6× sodium chloride/sodium citrate (SSC) at about 45° C, followed by a wash of 2×SSC at 50° C, are known or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. “Stringent hybridization conditions” are those that include a salt concentration of 1.0 M NaCl in 50% formamide, at a temperature of 37 °C for 4 to 12 hours, followed by a wash in 0.1X SSC at 60-65 °C.

[0071] As will be appreciated by the skilled practitioner, slight changes in nucleic acid sequence do not necessarily alter the amino acid sequence of the encoded polypeptide. This disclosure embraces the degeneracy of codon usage as would be understood by one of ordinary skill in the art. For example, as known in the art, different codons will code for the same amino acid as illustrated in the following chart.

<u>Amino Acid</u>	<u>Codons</u>
Ala/A	GCT, GCC, GCA, GCG
Arg/R	CGT, CGC, CGA, CGG, AGA, AGG

Asn/N	AAT, AAC
Asp/D	GAT, GAC
Cys/C	TGT, UGC
Gln/Q	CAA, CAG
Glu/E	GAA, GAG
Gly/G	GGT, GGC, GGA, GGG
His/H	CAT, CAC
Ile/I	ATT, ATC, ATA
Leu/L	TTA, TTG, CTT, CTC, CTA, CTG
Lys/K	AAA, AAG
Met/M	ATG
Phe/F	TTT, TTC
Pro/P	CCT, CCC, CCA, CCG
Ser/S	TCT, TCC, TCA, TCG, AGT, AGC
Thr/T	ACT, ACC, ACA, ACG
Trp/W	TGG
Tyr/Y	TAT, TAC
Val/V	GTT, GTC, GTA, GTG
START	ATG
STOP	TAG, TGA, TAA

[0072] The phrase “codon degenerate variant” when used with reference to a nucleic acid sequence means a nucleic acid sequence that differs from the referenced sequence, but that encodes a polypeptide having the same amino acid sequence as that encoded by the referenced sequence.

[0073] Additionally, it will be appreciated by persons skilled in the art that partial sequences often work as effectively as full-length versions. The ways in which the nucleotide sequence can be varied

or shortened are well known to persons skilled in the art, as are ways of testing the suitability or effectiveness of the altered genes. In certain embodiments, suitability and/or effectiveness of the altered gene may easily be tested by, for example, conventional gas chromatography. All such variations of the genes are therefore included as part of the present disclosure.

[0074] The term “isolated” and its grammatical equivalents refer to the removal of a nucleic acid from its natural environment. It is to be understood, however, that nucleic acids and proteins can be formulated with diluents or adjuvants and still for practical purposes be isolated.

[0075] The term “purified” and its grammatical equivalents refer to a molecule or composition, whether removed from nature (including genomic DNA and mRNA) or synthesized (including cDNA) and/or amplified under laboratory conditions, that has been increased in purity, wherein “purity” is a relative term, not “absolute purity.” For example, nucleic acids typically are mixed with an acceptable carrier or diluent when used for introduction into cells. The term “substantially purified” and its grammatical equivalents refer to a nucleic acid sequence, polypeptide, protein or other compound that is essentially free, *i.e.*, is more than about 50% free of, more than about 70% free of, more than about 90% free of, the polynucleotides, proteins, polypeptides and other molecules that the nucleic acid, polypeptide, protein or other compound is naturally associated with.

[0076] “Transposon,” “transposable element” or “TE” refers to a DNA sequence that can change its position within the genome, sometimes creating or reversing mutations and altering the cell’s genome size. Transposition often results in duplication of the transposon. Class I transposons are copied in two stages: first, they are transcribed from DNA to RNA, and the RNA produced is then reverse transcribed to DNA. This copied DNA is then inserted at a new position into the genome. The reverse transcription step is catalyzed by a reverse transcriptase, which can be encoded by the transposon itself. The characteristics of retrotransposons are similar to retroviruses, such as HIV. The cut-and-paste transposition mechanism of class II transposons does not involve an RNA intermediate. The transpositions are catalyzed by several transposase enzymes. Some transposases non-specifically bind to any target site in DNA, whereas others bind to specific DNA sequence targets. The transposase makes a staggered cut at the target site resulting in single-strand 5’ or 3’ DNA overhangs (sticky ends). This step cuts out the DNA transposon, which is then ligated into a new target site; this process involves activity of a DNA polymerase that fills in gaps and of a DNA ligase that closes the sugar-phosphate backbone. This results in duplication of the target site. The insertion sites of DNA transposons can be identified by short direct repeats which can be created by the staggered cut in the

target DNA and filling in by DNA polymerase, followed by a series of inverted repeats important for the transposon excision by transposase. Cut-and-paste transposons can be duplicated if their transposition takes place during S phase of the cell cycle when a donor site has already been replicated, but a target site has not yet been replicated. Transposition can be classified as either “autonomous” or “non-autonomous” in both Class I and Class II transposons. Autonomous transposons can move by themselves while non-autonomous transposons require the presence of another transposon to move. This is often because non-autonomous transposons lack transposase (for class II) or reverse transcriptase (for class I).

[0077] “Transposase” refers an enzyme that binds to the end of a transposon and catalyzes the movement of the transposon to another part of the genome by a cut and paste mechanism or a replicative transposition mechanism. In some embodiments, the transposase’s catalytic activity can be utilized to move gene(s) from a vector to the genome.

[0078] An “expression vector” or “vector” is any genetic element, *e.g.*, a plasmid, a mini-circle, a nanoplasmid, chromosome, virus, transposon, behaving either as an autonomous unit of polynucleotide replication within a cell (*i.e.* capable of replication under its own control) or being rendered capable of replication by insertion into a host cell chromosome, having attached to it another polynucleotide segment, so as to bring about the replication and/or expression of the attached segment. Suitable vectors include, but are not limited to, plasmids, transposons, bacteriophages and cosmids. Vectors can contain polynucleotide sequences that are necessary to effect ligation or insertion of the vector into a desired host cell and to effect the expression of the attached segment. Such sequences differ depending on the host organism; they include promoter sequences to effect transcription, enhancer sequences to increase transcription, ribosomal binding site sequences and transcription and translation termination sequences. Alternatively, expression vectors can be capable of directly expressing nucleic acid sequence products encoded therein without ligation or integration of the vector into host cell DNA sequences. In some embodiments, the vector is an “episomal expression vector” or “episome,” which is able to replicate in a host cell, and persists as an extrachromosomal segment of DNA within the host cell in the presence of appropriate selective pressure (see, *e.g.*, Conese et al., *Gene Therapy*, 11:1735-1742 (2004)). Representative commercially-available episomal expression vectors include, but are not limited to, episomal plasmids that utilize Epstein Barr Nuclear Antigen 1 (EBNA1) and the Epstein Barr Virus (EBV) origin of replication (oriP). The vectors pREP4, pCEP4, pREP7, and pcDNA3.1 from Invitrogen (Carlsbad, Calif.) and pBK-CMV from Stratagene (La Jolla, Calif.) represent non-limiting examples of an episomal vector that uses T-antigen and the SV40 origin

of replication in lieu of EBNA1 and oriP. A vector also can comprise a selectable marker gene. In certain embodiments where nanoplasmids are utilized, strains such as R6K that utilizes an antisense RNA selection marker (e.g. sucrose tolerance) can be used.

[0079] The term “selectable marker gene” refers to a nucleic acid sequence that allows cells expressing the nucleic acid sequence to be specifically selected for or against, in the presence of a corresponding selective agent. Suitable selectable marker genes are known in the art and described in, e.g., International Patent Application Publications WO 1992/08796 and WO 1994/28143; Wigler et al., Proc. Natl. Acad. Sci. USA, 77: 3567 (1980); O’Hare et al., Proc. Natl. Acad. Sci. USA, 78: 1527 (1981); Mulligan & Berg, Proc. Natl. Acad. Sci. USA, 78: 2072 (1981); Colberre-Garapin et al., J. Mol. Biol., 150:1 (1981); Santerre et al., Gene, 30: 147 (1984); Kent et al., Science, 237: 901-903 (1987); Wigler et al., Cell, 11: 223 (1977); Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA, 48: 2026 (1962); Lowy et al., Cell, 22: 817 (1980); and U.S. Pat. Nos. 5,122,464 and 5,770,359.

[0080] The term “coding sequence” refers to a segment of a polynucleotide that encodes for protein or polypeptide. The region or sequence is bounded nearer the 5’ end by a start codon and nearer the 3’ end with a stop codon. Coding sequences can also be referred to as open reading frames.

[0081] The term “operably linked” refers to refers to the physical and/or functional linkage of a DNA segment to another DNA segment in such a way as to allow the segments to function in their intended manners. A DNA sequence encoding a gene product is operably linked to a regulatory sequence when it is linked to the regulatory sequence, such as, for example, promoters, enhancers and/or silencers, in a manner, that allows modulation of transcription of the DNA sequence, directly or indirectly. For example, a DNA sequence is operably linked to a promoter when it is ligated to the promoter downstream with respect to the transcription initiation site of the promoter and in the correct reading frame with respect to the transcription initiation site to allow transcription elongation to proceed through the DNA sequence. An enhancer or silencer is operably linked to a DNA sequence coding for a gene product when it is ligated to the DNA sequence in such a manner as to, respectively, increase or decrease the transcription of the DNA sequence. Enhancers and silencers can be located upstream or downstream of or embedded within the coding regions of the DNA sequence. A DNA for a signal sequence is operably linked to DNA coding for a polypeptide if the signal sequence is expressed as a pre-protein that participates in the secretion of the polypeptide. Linkage of DNA sequences to regulatory sequences is typically accomplished by ligation at suitable restriction sites or via adapters or linkers inserted in the sequence using restriction endonucleases known to one of skill

in the art.

[0082] The terms “induce,” “induction” and their grammatical equivalents refer to an increase in nucleic acid sequence transcription, promoter activity and/or expression brought about by a transcriptional regulator, relative to some basal level of transcription.

[0083] The term “transcriptional regulator” refers to a biochemical element that acts to prevent or inhibit the transcription of a promoter-driven DNA sequence under certain environmental conditions (*e.g.*, a repressor or nuclear inhibitory protein), or to permit or stimulate the transcription of the promoter-driven DNA sequence under certain environmental conditions (*e.g.*, an inducer or an enhancer).

[0084] The term “enhancer” refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, *e.g.*, depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream or downstream of coding sequences or within coding sequences. The term “Ig enhancers” refers to enhancer elements derived from enhancer regions mapped within the immunoglobulin (Ig) locus (such enhancers include for example, the heavy chain (μ) 5' enhancers, light chain (κ) 5' enhancers, κ and μ intronic enhancers, and 3' enhancers (see generally Paul W. E. (ed), *Fundamental Immunology*, 3rd Edition, Raven Press, New York (1993), pages 353-363; and U.S. Pat. No. 5,885,827).

[0085] The term “promoter” refers to a region of a polynucleotide that initiates transcription of a coding sequence. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5' region of the sense strand). Some promoters are constitutive as they are active in all circumstances in the cell, while others are regulated becoming active in response to specific stimuli, *e.g.*, an inducible promoter. The term “promoter activity” and its grammatical equivalents refer to the extent of expression of nucleotide sequence that is operably linked to the promoter whose activity is being measured. Promoter activity can be measured directly by determining the amount of RNA transcript produced, for example by Northern blot analysis or indirectly by determining the amount of product coded for by the linked nucleic acid sequence, such

as a reporter nucleic acid sequence linked to the promoter.

[0086] “Inducible promoter” refers to a promoter that is induced into activity by the presence or absence of transcriptional regulators, *e.g.*, biotic or abiotic factors. Inducible promoters are useful because the expression of genes operably linked to them can be turned on or off at certain stages of development of an organism or in a particular tissue. Non-limiting examples of inducible promoters include alcohol-regulated promoters, tetracycline-regulated promoters, steroid-regulated promoters, metal-regulated promoters, pathogenesis-regulated promoters, temperature-regulated promoters and light-regulated promoters. The inducible promoter can be part of a gene switch or genetic switch.

[0087] The phrase “functional fragment” when used with reference to a polypeptide refers to a fragment of such polypeptide that possesses the primary function of the referenced polypeptide. For example, a functional fragment of a polypeptide that serves as a transmembrane domain is a fragment of that polypeptide that also serves as a transmembrane domain. In certain embodiments, the functional fragment of a polypeptide is shorter than the referenced polypeptide by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. When used with reference to a nucleic acid, the phrase “functional fragment” refers to a fragment of the referenced nucleic acid that encodes a polypeptide having the same primary function as the polypeptide encoded by the referenced nucleic acid.

[0088] The phrase “functional variant” when used with reference to a polypeptide refers to a polypeptide that differs from the referenced polypeptide but possesses the primary function of the referenced polypeptide. For example, a functional variant of a polypeptide that serves as a transmembrane domain is a fragment of that polypeptide that also serves as a transmembrane domain. In certain embodiments, the functional variant has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the referenced amino acid sequence and/or is a conservatively-substituted variant of the referenced sequence. In certain embodiments, the functional variant is a conservatively substituted variant of the referenced sequence that only differs therefrom by 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 conservative amino acid substitution(s).

[0089] When used with reference to a nucleic acid, the phrase “functional variant” refers to a nucleic acid that differs from the referenced nucleic acid but encodes a polypeptide having the same primary function as the polypeptide encoded by the referenced nucleic acid. In certain embodiments, the functional variant has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

or 99% sequence identity with the referenced nucleic acid sequence, hybridizes under stringent hybridization conditions with the complement of the referenced nucleic acid sequence, or is a codon degenerate variant of the nucleic acid sequence.

[0090] “Patient” or “subject” refers to a mammalian subject diagnosed with or suspected of having or developing a disease or disorder in connection with alpha-1 antitrypsin deficiency, such as liver disease or lung disease. In some embodiments, the term “patient” refers to a mammalian subject with a higher than average likelihood of developing a disease such as lung disease or liver disease attributed to alpha-1 antitrypsin deficiency. Exemplary patients can be humans, apes, dogs, pigs, cattle, cats, horses, goats, sheep, rodents and other mammals that can benefit from the therapies disclosed herein. Exemplary human patients can be male and/or female. “Patient in need thereof” or “subject in need thereof” means a patient diagnosed with or suspected of having a disease or disorder, for instance, but not restricted to lung disease or liver disease.

[0091] “Administering” refers to providing one or more compositions described herein to a patient or a subject. By way of example and not limitation, composition administration, *e.g.*, injection, can be performed by intravenous (i.v.) injection (such as portal vein delivery), sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, or intramuscular (i.m.) injection. One or more such routes can be employed. Parenteral administration can be, for example, by bolus injection or by gradual perfusion over time. Alternatively, or concurrently, administration can be by the oral route. Additionally, administration can also be by surgical deposition of a bolus or pellet of cells, or positioning of a medical device.

[0092] The terms “treatment,” “treating,” and their grammatical equivalents refer to obtaining a desired pharmacologic and/or physiologic effect. In some embodiments, the effect is therapeutic, *i.e.*, the effect partially or completely cures a disease and/or adverse symptom attributable to the disease. In some embodiments, the term “treating” can include “preventing” a disease or a condition.

[0093] A “treatment interval” refers to a treatment cycle, for example, a course of administration of a therapeutic agent that can be repeated, *e.g.*, on a regular schedule. In some embodiments, a dosage regimen can have one or more periods of no administration of the therapeutic agent in between treatment intervals.

[0094] The terms “administered in combination,” “co-administration,” “co-administered,” “co-administering,” and “co-providing” mean that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, *e.g.*, the two or more treatments

are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[0095] In some embodiments, the first treatment and second treatment can be administered simultaneously (e.g., at the same time), in the same or in separate compositions, or sequentially. Sequential administration refers to administration of one treatment before (e.g., immediately before, less than 5, 10, 15, 30, 45, 60 minutes; 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 48, 72, 96 or more hours; 4, 5, 6, 7, 8, 9 or more days; 1, 2, 3, 4, 5, 6, 7, 8 or more weeks before) administration of an additional, e.g., secondary, treatment. The order of administration of the first and secondary treatment can also be reversed.

[0096] The terms "therapeutically effective amount," "therapeutic amount," "immunologically effective amount," "anti-tumor effective amount," "tumor-inhibiting effective amount," and their grammatical equivalents refer to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of a composition described herein to elicit a desired response in one or more subjects. The precise amount of the compositions of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, extent of disease or disorder, and condition of the patient (subject).

[0097] Alternatively, the pharmacologic and/or physiologic effect of administration of one or more

compositions described herein to a patient or a subject of can be “prophylactic,” *i.e.*, the effect completely or partially prevents a disease or symptom thereof. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (*e.g.*, prevention of disease onset).

[0098] As used herein, terms used in the identification of biological moieties may include, or may not include, a dash “ – ” within the term. The presence or absence of a dash does not change the intended meaning or identification of the biological moiety. By way of illustration only, and without limitation to these biological moieties, each of the following paired terms (shown with/without a dash) indicate and identify the same biological entities: CCR-4/CCR4, CD-3/CD3, CD-4/CD4, CD-33/CD33, EGFR-2/EGFR2, FLT-1/FLT1, HER-1/HER1, HER-1t/HER1t, IL-12/IL12, IL-15/IL15, IL-15R α /IL15R α , MUC-1/MUC1, MUC-16/MUC16, ROR-1/ROR1, ROR-1R/ROR1R, TGF-Beta/TGFBeta, VEGF-1/VEGF1, VEGF-R2/VEGFR2.”

II. miRNA(s)

[0099] The terms “miR,” “mir” and “miRNA” are used to refer to microRNA, a class of small non-coding RNA molecules that are capable of affecting the expression of a gene (the “target gene”) by modulating the translation of messenger RNA transcribed therefrom (either increasing or decreasing the gene’s expression) and/or destabilizing such messenger RNA.

[0100] The term “primary miRNA,” abbreviated “pri-miRNA,” refers to an miRNA containing at least one RNA hairpin. The RNA hairpin(s) are cleaved from the pri-miRNA in the cell nucleus to form one or more precursor miRNAs (“pre-miRNAs”). This pre-miRNA is exported into the cytoplasm where the stem loop structure is cleaved to produce a double-stranded miRNA comprising a miRNA-5p strand from the former 5’ arm of the hairpin loop and a miRNA-3p strand from the former 3’ arm of the hairpin loop. The Argonaute protein then binds the double-stranded miRNA and one of the strands (either the miRNA-5p sequence or the miRNA-3p sequence) is released. The remaining bound strand becomes the “guide strand” (also referred to as a guide miRNA) whereas the released strand is known as the “passenger strand” (also referred to as a passenger miRNA) and preferably degrades. The guide strand then goes on to interact with the messenger RNA derived from the target gene, thus affecting its translation or stability. Once the guide miRNA interacts with the target mRNA, the RNA-inducing silencing complex (RISC, also known as microRNA ribonucleoprotein complex (miRNP)) is recruited to the complex to, for example, cleave the target mRNA into at least two pieces, destabilize the target mRNA by shortening its poly(A) tail, or reduce

translation of the target mRNA.

[0101] Both the miRNA-5p and miRNA-3p strand sequences will be referred to herein as “mature miRNA” sequences. The remaining portions of a pri-miRNA or pre-miRNA (the portion thereof 5’ to the miRNA-5p sequence, the portion thereof 3’ to the miRNA-3p sequence, and the stem loop sequence in between the miRNA-5p and miRNA-3p sequences) will be collectively referred to as miRNA backbone sequences. The term “5’ backbone sequence” will be used herein to refer to the backbone sequence that, in a pri- or pre-miRNA, is 5’ of the miRNA-5p sequence. The term “3’ backbone sequence” will be used herein to refer to the backbone sequence that, in a pri- or pre-miRNA, is 3’ of the miRNA-3p sequence. The term “loop sequence” refers to the backbone sequence that, in a pri- or pre-miRNA, is between the miRNA-5p and miRNA-3p sequences.

[0102] The term “miRNA,” unless otherwise indicated, refers generically to the mature, primary, and precursor forms of a particular microRNA and functional fragments and variants thereof.

[0103] The miRNAs can be non-naturally occurring. The terms “non-naturally occurring,” “non-natural,” “synthetic,” and “artificial,” as used to describe miRNA(s) herein, are used interchangeably and refer to an miRNA having a sequence that does not occur in nature.

[0104] The present invention relates in part to a ribonucleic acid comprising one or more non-natural pre-miRNA sequences, wherein at least one pre-miRNA sequence comprises a guide miRNA that inhibits the expression of a SERPINA1 allele encoding a mutant variant of alpha-1 antitrypsin (AAT), for example, AAT(Z), AAT(S), AAT(I), AAT(Siiyama), or AAT(M_{malton}). In certain embodiments, the RNA comprises more than two such non-natural pre-miRNA sequences, for example three, four, five, six, seven, eight, nine, ten, or more such sequences. It is understood that each guide miRNA may target the same or a different allele. In embodiments wherein two or more guide miRNAs target the same allele, such guide miRNAs may target the same or different regions of such gene. In some embodiments, the RNA comprises two or more non-natural pre-miRNA sequences, where at least one pre-miRNA sequence comprises a guide miRNA that inhibits the expression of AAT(Z) and a second pre-miRNA sequence comprises a guide miRNA that inhibits the expression of any AAT allele (e.g., AAT(M), AAT(Z), or AAT(S)).

[0105] In certain embodiments, each non-natural pre-miRNA sequence in the ribonucleic acid forms a stem-loop secondary structure that is distinct and non-complementary from that formed by a different non-natural pre-miRNA sequence in the ribonucleic acid. In certain embodiments, the non-natural pre-miRNA sequences have less than about 99%, less than about 95%, less than about 94%, less than

about 93%, less than about 92%, less than about 91%, less than about 90%, less than about 85%, less than about 80%, less than about 75%, less than about 70%, less than about 65%, less than about 60%, less than about 55%, less than about 55%, or less than about 50% sequence identity with each other. For example, the non-natural pre-miRNA sequences have from about 25% to about 99% sequence identity with each other.

[0106] In certain embodiments, the secondary structure of each non-natural pre-miRNA is sufficiently similar to that of a naturally-occurring pre-miRNA sequence so as to reduce or prevent cellular RNAi-based anti-pathogen toxicity. In certain such embodiments, the nucleic acid sequence of a non-natural pre-miRNA has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with that of a naturally-occurring pre-miRNA and/or can hybridize under stringent hybridization conditions with a naturally-occurring pre-miRNA. For example, the nucleic acid sequence of a non-natural pre-miRNA has from about 50% to about 100% sequence identity with that of a naturally-occurring pre-miRNA and/or can hybridize under stringent hybridization conditions with a naturally-occurring pre-miRNA.

[0107] In certain embodiments, the secondary structure of each pri-miRNA containing a non-natural pre-miRNA (hereinafter, a “non-natural pri-miRNA”) is sufficiently similar to that of a naturally-occurring pri-miRNA sequence so as to reduce or prevent cellular RNAi-based anti-pathogen toxicity. In certain such embodiments, the nucleic acid sequence of a non-natural pri-miRNA has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with that of a naturally-occurring pri-miRNA and/or can hybridize under stringent hybridization conditions with a naturally-occurring pri-miRNA. For example, the nucleic acid sequence of a non-natural pri-miRNA has from about 50% to about 100% sequence identity with that of a naturally-occurring pri-miRNA and/or can hybridize under stringent hybridization conditions with a naturally-occurring pri-miRNA.

[0108] The non-natural pre-miRNA of the present invention may be produced from a naturally-occurring pre-miRNA by removing the native mature miRNA sequences and replacing them with non-

native mature miRNA sequences wherein one of the sequences is capable of serving as a guide miRNA targeting a gene of interest.

[0109] In certain embodiments, each non-natural pre-miRNA comprises backbone sequences derived from a naturally-occurring pre-miRNA, for example from that present in mouse, rat, or human. In certain embodiments, the backbone sequences (the 3' backbone sequence, the 5' backbone sequence, and the loop sequence) of the non-natural pre-miRNA have at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the corresponding backbone sequences of a naturally-occurring pre-miRNA and/or can hybridize under stringent hybridization conditions with such corresponding backbone segments. In certain embodiments, the backbone segments of the non-natural pre-miRNA sequences are identical to the corresponding backbone segments of a naturally-occurring pre-miRNA. In certain embodiments, the naturally-occurring pre-miRNA is miR16, miR17, miR19, miR21, miR22, miR26a1, miR29b1, miR30a, miR122, miR126, miR133a1, miR142, miR150, miR155, miR181a, miR204, miR206, miR214, miR412, miR486, miR494, or miR1915. In certain embodiments, the naturally-occurring pre-miRNA is miR16, miR17, miR21, miR22, miR26a1, miR142, miR150, miR204, or miR206. In certain embodiments, the naturally-occurring pre-miRNA is miR16, miR17, miR21, miR22, miR204, or miR206. In certain embodiments, the naturally-occurring pre-miRNA is miR204 or miR206. In certain embodiments, the naturally-occurring pre-miRNA is miR16, miR17, miR22, miR29b, miR133a1, miR150, miR181a1, miR204, miR206, or miR486.

[0110] In certain embodiments, each non-natural pri-miRNA comprises backbone sequences derived from a naturally-occurring pri-miRNA, for example from that present in mouse, rat, or human. In certain embodiments, the backbone sequences (the 3' backbone sequence, the 5' backbone sequence, and the loop sequence) of the non-natural pri-miRNA have at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the corresponding backbone sequences of a naturally-occurring pri-miRNA and/or can hybridize under stringent hybridization conditions with such corresponding backbone segments. For example, the backbone sequences of the non-natural pri-

miRNA have from about 50% to 100% sequence identity with the corresponding backbone sequences of a naturally-occurring pri-miRNA and/or can hybridize under stringent hybridization conditions with such corresponding backbone segments. In certain embodiments, the backbone segments of the non-natural pri-miRNA sequences are identical to the corresponding backbone segments of a naturally-occurring pri-miRNA. In certain embodiments, the naturally-occurring pri-miRNA is miR16, miR17, miR19, miR21, miR22, miR26a1, miR29b1, miR30a, miR122, miR126, miR133a1, miR142, miR150, miR155, miR204, miR206, miR214, miR412, miR486, miR494, or miR1915. In certain embodiments, the naturally-occurring pre-miRNA is miR16, miR17, miR21, miR22, miR26a1, miR142, miR150, miR204, or miR206. In certain embodiments, the naturally-occurring pre-miRNA is miR16, miR17, miR21, miR22, miR204, or miR206. In certain embodiments, the naturally-occurring pre-miRNA is miR204 or miR206.

[0111] While the miRNA-5p and miRNA-3p sequences hybridize with each other, they are not necessarily exactly complementary. In the design of a non-naturally occurring miRNA, compensatory mutations can be made in the miRNA-5p and/or miRNA-3p sequences so as to maintain the RNA folding and free energy of the naturally-occurring miRNA. In certain embodiments, the sequence encoding the miRNA-3p sequence has at least about 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the complement to the sequence encoding the miRNA-5p sequence or is capable of hybridizing under stringent hybridization conditions with the sequence encoding the miRNA-5p sequence.

[0112] In certain embodiments, the two non-natural pre-miRNA sequences are separated from each other by at least about 1, at least about 2, at last about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, at least about 130, at least about 140, at least about 150, at least about 160, at least about 170, at least about 180, at least about 190, at least about 200, at least about 210, at least about 220, at least about 230, at least about 240, or at least about 250 nucleotides. In certain embodiments, the two non-natural pre-miRNA sequences are separated from each other by about 5 to 250 nucleotides, about 10 to 250 nucleotides, about 10 to 200 nucleotides, about 10 to 150 nucleotides, about 10 to 100 nucleotides, about 10 to 50 nucleotides, about 10 to 40 nucleotides, about 10 to 30 nucleotides, about 10 to 20 nucleotides, about 16 to 250 nucleotides, about 16 to 200 nucleotides, about 16 to 150 nucleotides, about 16 to 100 nucleotides, about 16 to 50 nucleotides,

about 16 to 40 nucleotides, about 16 to 30 nucleotides, about 16 to 20 nucleotides, about 20 to 200 nucleotides, about 20 to 150 nucleotides, about 20 to 100 nucleotides, about 20 to 50 nucleotides, about 20 to 45 nucleotides, about 20 to 40 nucleotides, about 20 to 35 nucleotides, about 20 to 30 nucleotides, about 20 to 25 nucleotides, about 30 to 200 nucleotides, about 30 to 150 nucleotides, about 30 to 100 nucleotides, about 30 to 50 nucleotides, or about 30 to 40 nucleotides. In certain embodiments, the two non-natural pre-miRNA sequences are separated from each other by at least about 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, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, or 250 nucleotides. In certain embodiments, the two non-natural pre-miRNA sequences are separated from each other by at least about 7 nucleotides. In certain embodiments, the two non-natural pre-miRNA sequences are separated from each other by at least about 10 nucleotides.

[0113] In certain embodiments, two non-natural pri-miRNA sequences adjoin each other with the 3' nucleotide of one pri-miRNA being directly bonded to the 5' nucleotide of another pri-miRNA. In such embodiments, the nucleotides separating the respective non-natural pre-miRNAs contained in each pri-miRNA form part of the pri-miRNA sequences.

[0114] In certain embodiments, the non-natural pre-miRNA comprises a mature miRNA sequence that is capable of binding to an mRNA and thereby interfering with the translation thereof and/or prompting its degradation. The mRNA may be produced from the expression of a target gene.

[0115] In certain embodiments, the target gene encodes a mutant variant of AAT. Thus, the pre-miRNA sequences inhibit the expression of the mutant AAT by targeting the gene expressing the same. In certain such embodiments, the mutant variant of AAT is AAT(Z), AAT(S), AAT(I), AAT(S_{iiyama}), or AAT(M_{malton}).

[0116] In certain embodiments, each non-natural pre-miRNA targets a different allele. In certain each non-natural pre-miRNA targets a different region of the same allele. In certain each non-natural pre-miRNA targets a different region of a different allele.

[0117] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets an allele encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0118] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene expressing AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene expressing AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene expressing AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0119] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the

ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets an allele encoding AAT(Z).

[0120] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z) and (b) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z) and (b) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets an allele encoding AAT(Z).

[0121] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets an allele encoding AAT(Z).

[0122] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene

encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets an allele encoding AAT(Z).

[0123] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets an allele encoding AAT(Z).

[0124] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets an allele encoding AAT(Z).

[0125] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets

the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0126] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0127] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets an allele encoding AAT(Z).

[0128] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising

backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0129] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets an allele encoding AAT(Z).

[0130] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets an allele encoding AAT(Z).

[0131] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0132] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0133] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets an allele encoding

AAT(Z).

[0134] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets an allele encoding AAT(Z).

[0135] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets an allele encoding AAT(Z).

[0136] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR142 and a

guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR21 and a guide miRNA that targets an allele encoding AAT(Z).

[0137] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0138] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0139] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide

miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets an allele encoding AAT(Z).

[0140] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0141] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0142] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises:

(a) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0143] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets an allele encoding AAT(Z).

[0144] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets an allele encoding AAT(Z).

[0145] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z);

and (b) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets an allele encoding AAT(Z).

[0146] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets an allele encoding AAT(Z).

[0147] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets an allele encoding AAT(Z).

[0148] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR26a1 and a guide miRNA that targets an allele encoding AAT(Z).

[0149] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0150] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding

AAT(Z).

[0151] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0152] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets an allele encoding AAT(Z).

[0153] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16

and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0154] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein;

(b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0155] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from

miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0156] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the

ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR150 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0157] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein;

(b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone

sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0158] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain

embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0159] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA

comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0160] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT

allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0161] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain

embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences

from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0162] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene

encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR16 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR22 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0163] In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a

guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0164] In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising

backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the mutant gene encoding AAT(Z). In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the gene encoding any AAT allele described herein; and (c) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pri-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pri-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pri-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein. In certain embodiments, the ribonucleic acid comprises: (a) a pre-miRNA comprising backbone sequences from miR204 and a guide miRNA that targets the gene encoding any AAT allele described herein; (b) a pre-miRNA comprising backbone sequences from miR206 and a guide miRNA that targets the mutant gene encoding AAT(Z); and (c) a pre-miRNA comprising backbone sequences from miR17 and a guide miRNA that targets the gene encoding any AAT allele described herein.

[0165]

[0166] The present invention also relates in part to a deoxyribonucleic acid encoding any of the aforementioned ribonucleic acids.

[0167] Examples of deoxyribonucleic acid sequences that encode backbone sequences that may be used in the practice of the present invention include, but are not limited, to those listed in Table 1 below. The symbols of “X” and “Y” in Table 1 indicate nucleic acid sequences encoding, respectively, the guide miRNA (which may be either miRNA-5p or miRNA-3p) and the passenger miRNA (which may be either miRNA-5p or miRNA-3p), whereas the symbol of “n” indicates the number of nucleotides in such sequences, for example 16–30, preferably 18–25. In some embodiments, n can be 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 or 35 nucleotides. In certain embodiments, the deoxyribonucleic acids encoding backbone sequences are those that hybridize under stringent hybridization conditions with the complement of any one of the sequences listed in Table 1.

Table 1: Deoxyribonucleic acid sequences encoding miRNA backbone sequences

Pri-miRNA from which backbone sequences are derived	Nucleic acid sequences encoding 5' Backbone Sequence – X_n-Stem Loop Sequence – Y_n-3' Backbone Sequence
miR204	SEQ ID NO: 1- <u>X_n</u> -SEQ ID NO: 2- <u>Y_n</u> -SEQ ID NO: 3
miR206	SEQ ID NO: 4- <u>Y_n</u> -SEQ ID NO: 5- <u>X_n</u> -SEQ ID NO: 6
miR17	SEQ ID NO: 7- <u>X_n</u> -SEQ ID NO: 8- <u>Y_n</u> -SEQ ID NO: 9
miR150	SEQ ID NO: 10- <u>X_n</u> -SEQ ID NO: 11- <u>Y_n</u> -SEQ ID NO: 12
miR150	SEQ ID NO: 13- <u>Y_n</u> -SEQ ID NO: 14- <u>X_n</u> -SEQ ID NO: 15
miR16	SEQ ID NO: 16- <u>X_n</u> -SEQ ID NO: 17- <u>Y_n</u> -SEQ ID NO: 18
miR30a	SEQ ID NO: 19- <u>X_n(or Y_n)</u> -SEQ ID NO: 20- <u>Y_n(or X_n)</u> -SEQ ID NO: 21
miR126	SEQ ID NO: 22- <u>Y_n</u> -SEQ ID NO: 23- <u>X_n</u> -SEQ ID NO: 24
miR122	SEQ ID NO: 25- <u>X_n</u> -SEQ ID NO: 26- <u>Y_n</u> -SEQ ID NO: 27
miR214	SEQ ID NO: 28- <u>Y_n</u> -SEQ ID NO: 29- <u>X_n</u> -SEQ ID NO: 30
miR214	SEQ ID NO: 31- <u>X_n</u> -SEQ ID NO: 32- <u>Y_n</u> -SEQ ID NO: 33
miR29b1	SEQ ID NO: 34- <u>Y_n</u> -SEQ ID NO: 35- <u>X_n</u> -SEQ ID NO: 36

miR29b1	SEQ ID NO: 37- <u>X_n</u> -SEQ ID NO: 38- <u>Y_n</u> -SEQ ID NO: 39
miR133a1	SEQ ID NO: 40- <u>Y_n</u> -SEQ ID NO: 41- <u>X_n</u> -SEQ ID NO: 42
miR26a1	SEQ ID NO: 43- <u>X_n</u> -SEQ ID NO: 44- <u>Y_n</u> -SEQ ID NO: 45
miR412	SEQ ID NO: 46- <u>X_n</u> -SEQ ID NO: 47- <u>Y_n</u> -SEQ ID NO: 48
miR19	SEQ ID NO: 49- <u>X_n</u> -SEQ ID NO: 50- <u>Y_n</u> -SEQ ID NO: 51
miR21	SEQ ID NO: 52- <u>X_n</u> -SEQ ID NO: 53- <u>Y_n</u> -SEQ ID NO: 54
miR142	SEQ ID NO: 55- <u>X_n</u> -SEQ ID NO: 56- <u>Y_n</u> -SEQ ID NO: 57
miR494	SEQ ID NO: 58- <u>X_n</u> -SEQ ID NO: 59- <u>Y_n</u> -SEQ ID NO: 60
miR1915	SEQ ID NO: 61- <u>X_n</u> -SEQ ID NO: 62- <u>Y_n</u> -SEQ ID NO: 63
miR22	SEQ ID NO: 338- <u>Y_n</u> -SEQ ID NO: 339- <u>X_n</u> -SEQ ID NO: 340
miR26a1	SEQ ID NO: 341- <u>X_n</u> -SEQ ID NO: 342- <u>Y_n</u> -SEQ ID NO: 343
miR486	SEQ ID NO: 344- <u>X_n</u> -SEQ ID NO: 345- <u>Y_n</u> -SEQ ID NO: 346
miR181a	SEQ ID NO: 597- <u>X_n</u> -SEQ ID NO: 598- <u>Y_n</u> -SEQ ID NO: 599

[0168] In any of the foregoing embodiments, the sequence encoding the pre-miRNA may comprise:

SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively;

SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively;

SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9, respectively;

SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, respectively;

SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15, respectively;

SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18, respectively;

SEQ ID NO: 19, SEQ ID NO: 20, and SEQ ID NO: 21, respectively;

SEQ ID NO: 22, SEQ ID NO: 23, and SEQ ID NO: 24, respectively;

SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, respectively;

SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30, respectively;

SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33, respectively;
SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36, respectively;
SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39, respectively;
SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42, respectively;
SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45, respectively;
SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48, respectively;
SEQ ID NO: 49, SEQ ID NO: 50, and SEQ ID NO: 51, respectively;
SEQ ID NO: 52, SEQ ID NO: 53, and SEQ ID NO: 54, respectively;
SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57, respectively;
SEQ ID NO: 58, SEQ ID NO: 59, and SEQ ID NO: 60, respectively;
SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63, respectively;
SEQ ID NO: 338, SEQ ID NO: 339, and SEQ ID NO: 340, respectively;
SEQ ID NO: 341, SEQ ID NO: 342, and SEQ ID NO: 343, respectively;
SEQ ID NO: 344, SEQ ID NO: 345, and SEQ ID NO: 346, respectively;
SEQ ID NO: 597, SEQ ID NO: 598, and SEQ ID NO: 599, respectively;
SEQ ID NO: 589; or sequences having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with any of the foregoing sequences, or that are capable of hybridizing under stringent hybridization conditions to the complements of such sequences.

[0169] In certain embodiments, the dual miRNA of the present invention comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 589 (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 589 or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 589).

[0170] Non-limiting examples of nucleic acid sequences encoding the guide miRNA targeting genes encoding mutant AAT variants are listed in Table 2. Table 2 also lists the sequences encoding the passenger strand. As previously discussed, the guide and passenger strand are not necessarily

complementary. It is contemplated that the passenger strand may also serve to target the messenger RNA associated with the target gene. It is also contemplated that sequences that hybridize under stringent hybridization conditions with the compliments of the sequences listed in Table 2 may also be used. The mature miRNA sequences used may be combined with a specific pri-miRNA backbone. Table 2 also lists backbones that can be combined with the mature guide and passenger miRNAs listed therein.

Table 2: Deoxyribonucleic acid sequences encoding mature miRNA sequences

miRNA Target	Backbone	DNA encoding guide miRNA	DNA encoding passenger miRNA
AAT(Z)	miR-204	(SEQ ID NO: 64) ACGCTCTTCAGATCATAGGCCT	(SEQ ID NO: 65) AGGCTGTGAACTGAAGAG CGC
AAT(Z)	miR-206	(SEQ ID NO: 66) TTCTTGTCGATGGTCAGCATGG	(SEQ ID NO: 67) TGTTGACCGTCGACATTG AA

[0171] In certain embodiments, the present invention relates to a deoxyribonucleic acid comprising a nucleic acid sequence having at least about 80% sequence identity with any one of SEQ ID NOs: 64–67, or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 64–67. In certain such embodiments, the present invention relates to a deoxyribonucleic acid comprising a nucleic acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 64–67, or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 64–67.

[0172] In certain embodiments, the sequence encoding the guide miRNA sequence has at least about 80%, 85%, 90%, %, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 64 or 66, or is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 64 or 66.

[0173] In certain embodiments, the sequence encoding the passenger miRNA sequence has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to

SEQ ID NO: 65 or 67, or is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 65 or 67.

[0174] In certain embodiments, the present invention relates to a deoxyribonucleic acid wherein each sequence encoding a pre-miRNA comprises:

- a) a sequence encoding a 5' miRNA backbone sequence;
- b) a sequence encoding a guide miRNA sequence;
- c) a sequence encoding a stem loop sequence;
- d) a sequence encoding a passenger miRNA sequence; and
- e) a sequence encoding a 3' backbone sequence.

[0175] In certain embodiments, the sequence encoding the pre-miRNA comprises:

- a) a guide miRNA sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity SEQ ID NO: 64 or 66, or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 64 or 66; and
- b) a passenger sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 65 or 67, or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 65 or 67.

[0176] Deoxyribonucleic acids encoding exemplary non-natural pre-miRNA sequences targeting AAT(Z) are described in Table 3. In certain embodiments, the deoxyribonucleic acid may comprise a sequence that is capable of hybridizing under stringent hybridization conditions with the complement of any one of the sequences listed in Table 3.

Table 3: Deoxyribonucleic acid sequences encoding non-natural miRNA sequences

miRNA Target	Backbone sequences derived from	DNA Sequence Encoding pri-miRNA Sequence	DNA Sequence Encoding pre-miRNA Sequence
AAT(Z)	miR204	(SEQ ID NO: 178) AGGAGGGTGGGG GTGGAGGCAAGCA GAGGACTTCCTGA TCGCGTACCCATG GCTACAGTCTTTCT TCATGTGACTCGT GGACACGCTCTTC AGATCATAGGCCT GAGAATATATGAA GGAGGCTGTGAAC TGAAGAGCGCGTT CAATTGTCATCAC TGGCATCTTTTTTG ATCATTGCACCAT CATCAAATGCATT GGGATAACCATGA C	(SEQ ID NO: 347) GGCTACAGTCTTT CTTCATGTGACTC GTGGACACGCTCT TCAGATCATAGGC CTGAGAATATATG AAGGAGGCTGTGA ACTGAAGAGCGCG TTCAATTGTCATC ACTGGC
AAT(Z)	miR206	(SEQ ID NO: 179) GATGCTACAAGTG GCCCCACTTCTGAG ATGCGGGCTGCTT CTGGATGACACTG CTTCCCGAGGCCA TGTTGACCGTCGA CATTGAATATGGA	(SEQ ID NO: 348) TGCTTCCCGAGGC CATGTTGACCGTC GACATTGAATATG GATTACTTTGCTAT TCTTGTCGATGGT CAGCATGGTTTCG GCAAGTG

miRNA Target	Backbone sequences derived from	DNA Sequence Encoding pri-miRNA Sequence	DNA Sequence Encoding pre- miRNA Sequence
		TTACTTTGCTATTC TTGTCGATGGTCA GCATGGTTTCGGC AAGTGCCTCCTCG CTGGCCCCAGGGT ACCACCCGGAGCA CAGGTTTGGTGAC CTT	

[0177] In certain embodiments, the present invention relates to a deoxyribonucleic acid comprising a sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 178 or 179 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 178 or 179.

[0178] In certain embodiments, the present invention relates to a deoxyribonucleic acid comprising a sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 347 or 348 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 347 or 348.

[0179] In embodiments of the present invention, the two or more pre-miRNAs encoded by the deoxyribonucleic acid may each contain guide miRNA sequences that target the same target allele or the various guide miRNAs may target different alleles. In addition, each pre-miRNA design of that of the pri-miRNA containing them may be based on a different naturally-occurring miRNA backbone to reduce the likelihood of misfolding of one miRNA with another. Table 4 provides examples of deoxyribonucleic acid sequences encoding two or more pri-miRNAs.

Table 4: Deoxyribonucleic acid sequences comprising two or more pri-miRNAs

miRNA Target	Backbone sequences derived from	DNA Sequence
Any AAT allele + AAT(Z)	miR204 + miR206	(SEQ ID NO: 267) AGGAGGGTGGGGGTGGAGGCAAGCAGAGGA CTTCCTGATCGCGTACCCATGGCTACAGTCTT TCTTCATGTGACTCGTGGACACGCTCTTCAGA TCATAGGCCTGAGAATATATGAAGGAGGCTG TGAAGTGAAGAGCGCGTTCAATTGTCATCAC TGGCATCTTTTTTGATCATTGCACCATCATCA AATGCATTGGGATAACCATGACGATGCTACA AGTGGCCCACTTCTGAGATGCGGGCTGCTTC TGGATGACACTGCTTCCCGAGGCCATGTTGA CCGTCGACATTGAATATGGATTACTTTGCTAT TCTTGTCGATGGTCAGCATGGTTTCGGCAAGT GCCTCCTCGCTGGCCCCAGGGTACCACCCGG AGCACAGGTTTGGTGACCTT

[0180] In certain such embodiments, the present invention relates to a deoxyribonucleic acid comprising a nucleic acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 267 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 267.

III. Protein of Interest

[0181] In certain embodiments, the deoxyribonucleic acid encoding the pre-miRNAs is contained in the same genetic construct as that comprising one or more genes encoding protein(s) of interest, for example, normal, functional alpha-1 antitrypsin encoded by AAT(M).

A. AAT(M)

[0182] In any of the foregoing embodiments, the protein of interest may be normal, functional alpha-1 antitrypsin ("AAT"). A genetic construct of the present invention encoding pre-miRNAs and encoding normal, functional AAT allows for therapy involving the use of miRNA to reduce expression

of the mutant variant(s) of AAT, such as AAT(Z), while introducing a transgene that expresses AAT(M). By combining the reduction of mutant AAT expression with the introduction of functional AAT, such gene therapy offers a comprehensive approach to address the underlying cause of AATD. In certain embodiments, the transgene encoding AAT(M) differs from the wild-type gene in that it is modified to have silent mutations to evade reduction of expression by certain miRNA constructs contemplated herein, e.g., to evade reduction of expression by miRNA constructs designed to reduce expression of mutant AAT alleles, such as AAT(Z). In certain such embodiments, the miRNA-resistant AAT(M) gene variant comprises the nucleic acid sequence of SEQ ID NO: 590 or a functional variant thereof. In certain such embodiments, the functional variant has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the SEQ ID NO: 590 and/or is a codon degenerate variant of SEQ ID NO: 590.

[0183] In certain embodiments, an AAT(M) variant is modified to reduce the presence of CpG sites and to evade reduction of expression by certain miRNA constructs as described above. In certain such embodiments, the CpG-reduced version of the miRNA-resistant AAT(M) gene variant comprises the nucleic acid sequence of SEQ ID NO: 596 or a functional variant thereof. In certain such embodiments, the functional variant has a least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the SEQ ID NO: 596 and/or is a codon degenerate variant of SEQ ID NO: 596. In certain embodiments, the functional variant is a codon degenerate variant of SEQ ID NO: 596.

[0184] In certain embodiments, the AAT(M) transgene encodes an amino acid sequence having at least about 80% sequence identity with SEQ ID NO: 600 (*e.g.*, an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 600), or a conservatively-substitute variant of SEQ ID NO: 600.

IV. Genetic Construct

[0185] As previously discussed, in certain embodiments, the deoxyribonucleic acid encoding the pre-miRNAs is contained in the same genetic construct as that comprising one or more genes encoding protein(s) of interest (*e.g.*, AAT(M) variant encoding wild-type AAT protein).

[0186] In a preferred embodiment, the genetic construct comprises at least the following: a (i) tissue-specific promoter (*e.g.*, a liver-specific promoter); (ii) a 5' UTR; (iii) any of the mutant AAT-targeting miRNA designs disclosed herein (*e.g.*, miR204 + miR206); (iv) silencer/miRNA-resistant AAT(M) transgene; and (v) a 3' UTR.

[0187] In yet another preferred embodiment, the genetic construct comprises the following: a (i) a liver-specific promoter; (ii) a 5' UTR; (iii) two of the mutant AAT-targeting miRNA designs disclosed herein (*e.g.*, miR204 + miR206); (iv) silencer/miRNA-resistant AAT(M) transgene; and (v) a 3' UTR. Notably, a key inventive aspect of this preferred embodiment is that only one of the two miRNAs specifically targets across the mutant AAT variant (*e.g.*, AAT(Z)). The second miRNA targets a different site that is shared across other alleles, including AAT(M). In certain embodiments, the genetic construct of the present invention comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 592 (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 592), a codon degenerate variant of SEQ ID NO: 592, or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 592.

[0188] In another embodiment, a vector is used to deliver the genetic construct to a subject or patient. In a further preferred embodiment, a gorilla adenovirus vector is used to deliver the genetic construct. Any genetic construct described herein can be inserted into a vector in either a forward or reverse orientation. In certain embodiments, the genetic construct is inserted into a gorilla adenovirus vector in a reverse orientation. In one such embodiment, the gorilla adenovirus vector is a GC44 vector comprising a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 593 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 593), a codon degenerate variant of SEQ ID NO: 593, or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 593.

[0189] In one embodiment, the deoxyribonucleic acid encoding the pre-miRNAs is contained in the same genetic construct as that comprising a gene variant (*i.e.*, AAT(M)) that is resistant to reduction of expression by miRNA and encodes normal, functional AAT protein. In one embodiment, the gene variant comprises at least one silent mutation or codon variant that allows the gene variant to avoid reduction of expression by miRNA and encode normal, functional AAT protein. In a further embodiment, the AAT(M) gene variant is miRNA-resistant and comprises the nucleic acid sequence of SEQ ID NO: 590 or a functional variant thereof. In certain such embodiments, the functional variant has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the SEQ ID NO: 590 and/or is a conservatively-substituted variant of SEQ ID NO: 590. In certain embodiments, the functional variant is a conservatively substituted variant of SEQ ID NO: 590 that only differs therefrom by 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or

fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 conservative nucleic acid base substitution(s).

[0190] In certain such embodiments, the genetic construct includes a nucleic acid sequence encoding a 5' untranslated region (5'UTR) directly upstream of a gene encoding a protein of interest and the pre-miRNA sequences are included in the 5'UTR. In one embodiment, the 5'UTR is from human GADPH. In a further embodiment, the 5'UTR comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 588 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 588).

[0191] In certain embodiments, such a genetic construct includes a nucleic acid sequence encoding a 3' untranslated region (3'UTR) directly downstream of a gene encoding a protein of interest and the pre-miRNA sequences are included in the 3'UTR. In one embodiment, the 3'UTR comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 591 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 591).

[0192] In certain embodiments, the genetic construct includes nucleic acid sequences encoding both a 5'UTR and a 3'UTR with each such region containing at least one pre-miRNA sequence (for example, each UTR can include one pre-miRNA, the 5'UTR can include one pre-miRNA and the 3'UTR can include 2 pre-miRNAs, the 5'UTR can include two pre-miRNAs and the 3'UTR can include one pre-miRNA, both UTRs can include two pre-miRNAs, etc.).

[0193] In embodiments wherein the sequence encoding the pri-miRNA is included in the sequence corresponding to the 5'UTR, the transcribed RNA can include additional sequences such as splice donor, branch point and/or acceptor site sequences. The inclusion of splice donor, branch point, and acceptor sites is important for splicing of the miRNAs from the transcribed RNA. Without splicing, the highly structured miRNA sequence is likely to impede ribosome scanning to the translation initiation sequence relating to the gene of interest. Examples of sequences encoding such splice donor/acceptor sites include SEQ ID NOs: 291 and 292, sequences having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with such sequences and sequences that are capable of hybridizing with the complement of such sequences under stringent hybridization conditions.

[0194] Thus, in certain embodiments, the deoxyribonucleic acids of the present invention further comprises: a) a nucleic acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 291 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 291; and b) a nucleic acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 292 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 292.

[0195] In certain embodiments, the portion of the deoxyribonucleic acid encoding a pre-miRNA is contained in a section corresponding to an intron within the gene encoding a protein of interest.

[0196] In other embodiments, at least one pre-miRNA is contained within the 5'UTR. In other embodiments, at least one pre-miRNA is contained in an intron located within the 5'UTR (hereinafter referred to as the "5'UTR intron"). In other embodiments, at least one pre-miRNA is contained within an intron located in the open reading frame. In other embodiments, at least one pre-miRNA is contained within the 3'UTR.

[0197] The polynucleotide of the present invention can include an integration signal for attP/attB phage integration of the polynucleotide into a human genome. The polynucleotide can further include a 5' homology arm or 5' terminal repeat and a 3' homology arm or 3' terminal repeat. The polynucleotide can further include insulators, boundary elements and S/MAR positioned 3' adjacent to the 5' homology arm or 5' terminal repeat and 5' adjacent to the 3' homology arm or 3' terminal repeat. Between the insulators, boundary elements, or S/MAR, the polynucleotide can include, from 5' to 3', a promoter which can include a silencer, enhancer, TF binding modules and a core promoter; a 5' untranslated region which can include stability modules, translation control elements, and intron-embedded elements such as miRNA encoding sequences; one or more genes which can include signal peptides, extracellular domains, transmembrane domains, signaling domains, antibody domains, peptide linkers, inteins and epitope tags; and a 3' untranslated region that can include stability modules, translation control, 3' end processing signals and a transcription terminator.

[0198] As previously discussed, the miRNA may be encoded in the same genetic construct with additional proteins of interest (*e.g.*, normal, functional AAT). Advantages of having two or more of such components expressed using one genetic construct include stoichiometric expression of such components, reduced product complexity, and reduced cost of production and administration.

[0199] As understood by those skilled in the art, genes encoding the polypeptides of interest may be linked by way of linkers. Any suitable linker known to link genes may be used in the practice of the present invention. Examples, of such linkers include those encoding an internal ribosome entry site

(IRES), cleavable peptides, and ribosomal skipping peptides. Examples cleavable peptides encoded by such linkers include Furinlink, fmdv, and 2A linkers (*e.g.*, P2A, GSG-P2A, FP2A, T2A, and Furin-T2A), or functional fragments or variants thereof.

[0200] The polynucleotide of the invention can be present in the construct in operable linkage with a promoter. Appropriate promoters can be selected based on the host cell and effect sought. Suitable promoters include constitutive and inducible promoters. The promoters can be tissue-specific, such promoters being well known in the art. *See, e.g.*, Zheng C, Baum BJ. *Methods Mol Biol.* 2008;434:205-219. Additionally, tissue-specific promoters can be synthetic tissue-specific promoters, such as a synthetic liver tissue specific promoter.

[0201] Examples of constitutive promoters for use in the present invention include, but are not limited to, immediate early cytomegalovirus (CMV) promoter; human elongation growth factor 1 alpha 1 (hEF1A1); simian virus 40 (SV40) early promoter; mouse mammary tumor virus (MMTV); human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter; MoMuLV promoter; avian leukemia virus promoter; Epstein-Barr virus immediate early promoter; Rous sarcoma virus promoter; and human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter; and functional fragments and variants thereof. In contrast to constitutive promoters, the use of an inducible promoter provides a molecular switch capable of turning on the expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to, a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as a RHEOSWITCH[®] gene switch.

Tissue-Specific Promoter

[0202] A novel aspect of the genetic construct described herein is the presence of a tissue-specific promoter. Tissue-specific DNA promoter sequences vary depending on the tissue or cell type they regulate. For example, a muscle-specific DNA promoter sequence is responsible for activating genes specifically in muscle cells. It contains specific DNA sequences that interact with muscle-specific transcription factors, such as MyoD, Myogenin, and MEF2 (myocyte enhancer factor 2). These transcription factors bind to the promoter sequence and recruit RNA polymerase, initiating the

transcription of muscle-specific genes. Similarly, other tissues or cell types, such as the brain, heart, kidney, lung, liver, or skin, have their own unique DNA promoter sequences that control gene expression in a tissue-specific manner. These promoter sequences often contain enhancer and silencer elements that fine-tune gene expression levels and respond to specific signals and factors present in the tissue microenvironment.

[0203] In one embodiment, the tissue-specific promoter is specific to the lung. In embodiments where the genetic construct of the present invention comprises a lung-specific promoter, transgene expression is restricted to lung tissue and the promoter provides high and sustained levels of transgene expression therein.

[0204] The genetic construct of the present invention may also include any of the following lung-specific promoters: surfactant protein B (SP-B) promoter, Clara cell secretory protein (CCSP) promoter, mucin promoter, and/or prosurfactant protein C (proSP-C) promoter.

[0205] In one embodiment, the genetic construct of the present invention comprises a surfactant protein B (SP-B) promoter. Surfactant Protein B is a protein involved in reducing surface tension in the lungs, allowing for proper lung function and gas exchange. The SP-B promoter is a lung-specific DNA promoter sequence that regulates the transcription of the SP-B gene specifically in lung cells and contains binding sites for lung-specific transcription factors, such as Nkx2.1 (also known as TTF-1 or thyroid transcription factor-1) and GATA6.

[0206] In another embodiment, the genetic construct of the present invention comprises a Clara cell secretory protein (CCSP) promoter. Clara cells are non-ciliated secretory cells present in the bronchiolar epithelium of the lungs. The CCSP promoter is a lung-specific promoter that drives the expression of the CCSP gene specifically in these Clara cells and contains binding sites for lung-specific transcription factors, including Nkx2.1 and Foxa2. The CCSP protein is involved in protecting the airways and modulating lung inflammation and repair.

[0207] In one embodiment, the genetic construct of the present invention comprises mucin promoters. Mucins are glycoproteins that play a crucial role in forming the mucus layer in the airways, which helps to protect and lubricate the respiratory system. Several mucin genes exhibit lung-specific expression. For example, MUC5AC and MUC5B are mucin genes that are predominantly expressed in the respiratory tract, including the lungs. The promoters of these genes contain lung-specific regulatory elements and are regulated by transcription factors such as Nkx2.1 and Spdef (SAM pointed domain-containing ETS transcription factor).

[0208] In yet another embodiment, the genetic construct of the present invention comprises a prosurfactant protein C (proSP-C) promoter. Prosurfactant protein C is a protein that is essential for the maintenance and stability of lung surfactant. The proSP-C promoter is a lung-specific promoter that drives the expression of the proSP-C gene predominantly in lung alveolar type II cells and contains binding sites for lung-specific transcription factors, including Nkx2.1 and Foxa2.

[0209] In another embodiment, the tissue-specific promoter is specific to the liver. In embodiments where the genetic construct of the present invention comprises a liver-specific promoter, transgene expression is restricted to the liver and the promoter provides high and sustained levels of transgene expression therein.

[0210] The genetic construct of the present invention may also include any of the following liver-specific promoters: albumin promoter, cytochrome P450 promoter, alpha-1-antitrypsin (AAT) promoter, and/or transthyretin (TTR) promoter.

[0211] The genetic construct of the present invention may also include one or more other types of regulatory sequences involved in the activation or regulation of liver-specific genes, such as: enhancers (e.g., liver-specific enhancer element of the albumin gene (Alb), alpha-fetoprotein enhancers; transthyretin (TTR) enhancers; cytochrome P450 family 3A (CYP3A) enhancers; and alpha-1 microglobulin/bikunin (A1-MB) enhancers); transcription factors (e.g., Hepatocyte Nuclear Factors (HNFs), particularly HNF1, HNF3, and HNF4); response elements (e.g., the CCAAT/enhancer-binding protein (C/EBP) response element); and silencers (e.g., DNA or RNA sequences that act as negative regulators of gene expression). In one embodiment, the liver-specific promoter comprises two A1-MB enhancers.

[0212] In one embodiment, the genetic construct of the present invention comprises the alpha-fetoprotein (AFP) promoter. AFP is a protein produced primarily in the liver during fetal development, and its expression decreases significantly after birth. The AFP promoter region contains specific DNA sequences that interact with liver-specific transcription factors, such as HNFs and C/EBP. These transcription factors bind to the promoter sequence and facilitate the recruitment of RNA polymerase, which initiates the transcription of the AFP gene.

[0213] In another embodiment, the genetic construct of the present invention comprises an albumin promoter. The albumin gene encodes the protein albumin, which is primarily synthesized in the liver. The albumin promoter enhances transcription of the albumin gene and contains binding sites for liver-specific transcription factors like HNF1 (hepatocyte nuclear factor 1) and HNF4. These transcription

factors bind to the albumin promoter, ensuring specific gene expression in liver cells.

[0214] In another embodiment, the genetic construct of the present invention comprises cytochrome P450 promoters. Cytochrome P450 (CYP) enzymes are a superfamily of enzymes involved in drug metabolism, hormone synthesis, and detoxification. Several members of the CYP family exhibit liver-specific expression. For example, the CYP3A4 promoter is active predominantly in liver cells and is regulated by a combination of liver-specific transcription factors such as HNF4 and CAR (constitutive androstane receptor). The CYP2E1 promoter is another liver-specific promoter that drives the expression of the CYP2E1 enzyme primarily in hepatocytes.

[0215] In yet another embodiment, the genetic construct of the present invention comprises a transthyretin (TTR) promoter: Transthyretin is a protein synthesized primarily in the liver and is involved in transporting thyroxine and retinol-binding protein. The TTR promoter drives the expression of the TTR gene specifically in liver cells and contains binding sites for liver-specific transcription factors like HNF1, HNF3, and HNF4. Mutations in the TTR promoter have been associated with hereditary forms of transthyretin amyloidosis, a condition characterized by the deposition of abnormal proteins in various organs.

[0216] In a preferred embodiment of the present invention, the genetic construct of the present invention comprises an alpha-1-antitrypsin (AAT) promoter. As disclosed earlier herein, alpha-1-antitrypsin is a protein produced in the liver and plays a role in protecting the lungs from damage caused by neutrophil elastase. In one embodiment, the AAT promoter is a liver-specific promoter that regulates the transcription of the AAT gene. In another embodiment, the AAT promoter contains binding sites for liver-specific transcription factors such as HNF1 and C/EBP.

[0217] The AAT promoter may be a partial promoter or full promoter. A full promoter, also known as a complete promoter, refers to a promoter region that contains all the necessary elements for efficient initiation of transcription. A partial promoter, on the other hand, refers to a promoter region that is incomplete or partially functional. It may lack some of the necessary elements or have mutations that impair its functionality. As a result, a partial promoter may have reduced or compromised activity in driving gene expression compared to a full promoter.

[0218] In the context of certain embodiments described herein, the liver-specific promoter is an AAT promoter that limits expression of AAT(M) to the liver region, or decreases expression of AAT(M) in organs other than the liver. In a further embodiment, the liver-specific promoter provides high and sustained levels of expression of AAT(M), especially relative to levels of expression of

AAT(M) expression under the control of a CMV promoter. In one embodiment, the genetic construct comprises a liver-specific promoter having at least 80% sequence identity with SEQ ID NO: 587 or a functional variant thereof (e.g., a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 587 or a nucleic acid capable of hybridizing under stringent hybridization conditions with the complement of SEQ ID NO: 587).

CpG Sites

[0219] It is also well understood in the art that promoters may be flanked by other distinctive DNA sequences to enhance or decrease transgene expression. For example, short interspersed DNA sequences, like CpG sites, that deviate significantly from the average genomic pattern may be used to control promoter function. Deaton AM, Bird A. *Genes Dev.* 2011;25(10):1010-1022.

[0220] “CpG” refers to a specific sequence of DNA nucleotides composed of a cytosine (C) followed by a guanine (G), connected by a phosphate group (p). CpG sites are often found in clusters known as CpG islands, which are regions of DNA that contain a high frequency of CpG dinucleotides.

[0221] In genetic engineering, CpG sites have particular significance in relation to gene expression and regulation. CpG sites, including CpG islands, are commonly found near the promoters of genes and can have an impact on gene expression. Normally, DNA methylation occurs at CpG sites, where a methyl group (CH₃) is added to the cytosine residue. Methylation of CpG sites in gene promoters is generally associated with gene silencing or reduced gene expression. The presence of methyl groups can interfere with the binding of transcription factors and other proteins required for gene transcription, leading to a decrease in gene activity. Accordingly, CpG sites may be manipulated to modulate gene expression. For example, the removal or deletion of CpG sites can be used to promote the expression of a gene of interest.

[0222] In certain embodiments, a genetic construct of the present invention is modified to reduce the presence of CpG sites. For example, any region of any of the vectors disclosed herein can be a CpG reduced version (e.g., backbone sections, promoter, untranslated regions, miRNAs, nucleic acids encoding AAT(M)). In certain embodiments, a GC44 vector containing the genetic construct of the present invention, a GC45 vector containing the genetic construct of the present invention, or a GC46 vector containing the genetic construct of the present invention is a CpG reduced version. In one embodiment, a promoter within the genetic construct is modified to reduce the presence of CpG sites. In a further embodiment, the promoter is a liver-specific promoter wherein the amount of CpG is reduced. In one embodiment, the CpG-reduced liver-specific promoter of the genetic construct

comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 594 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 594).

[0223] In addition to manipulating CpG sites, including CpG islands, near the promoters of genes to promote the expression of a gene of interest, the reduction of CpG sites also helps reduce potential immune activation. CpGs in plasmids or viral vectors are usually numerous and unmethylated, in contrast to mammalian CpGs, which tend to be methylated, are fewer overall, and more concentrated in/near promoters. The unmethylated CpGs in the viral vector are recognized in the cell by TLR9, thereby activating an immune response against the vector DNA. Accordingly, in the context of the present invention, CpGs within the genetic construct may be reduced to reduce potential, undesired immune activation.

[0224] In certain embodiments, the miRNA-resistant gene encoding AAT(M) previously disclosed herein is further modified to reduce the amount of CpG therein. In one embodiment, the CpG-reduced version of the miRNA-resistant AAT(M) gene variant comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 596 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 596), or a codon degenerate variant of SEQ ID NO: 596.

[0225] In certain embodiments, the 5'UTR of the genetic construct disclosed herein is modified to reduce the amount of CpG therein. In one embodiment, the CpG-reduced version of the 5'UTR comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 595 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 595), or a codon degenerate variant of SEQ ID NO: 595.

[0226] The present invention relates in part to a deoxyribonucleic acid comprising a genetic construct as described above.

V. Vectors and Delivery Systems

[0227] The ribonucleic acid and/or the deoxyribonucleic acid of the present invention can be delivered to cells on a long oligonucleotide, which is then inserted into a specific genome location. In certain embodiments, the ribonucleic acid and/or the deoxyribonucleic acid of the present invention

can be integrated into a cell's genome through gene editing systems that utilize CRISPR, TALEN or Zinc-Finger nucleases.

[0228] The polynucleotide of the present invention can be delivered to a target cell by any suitable delivery system, including non-viral and viral delivery systems. The present invention thus also relates in part to a vector comprising the ribonucleic acid or the deoxyribonucleic acid of the present invention.

[0229] Any vector known in the art for use in delivering ribonucleic acids (RNA) or deoxyribonucleic acids (DNA) may be used in the practice of the present invention. In certain embodiments, the vector is a plasmid, a mini-circle DNA, a nanoplasmid, a viral vector, an episomal vector, or a non-viral vector. Examples of viral vectors for use in the present invention include lentiviral vectors, retroviral vectors, adenovirus (Ad) vectors, adeno-associated virus (AAV) vectors (e.g., AAV1, AAV2, AAV7, AAV8, or AAV9), herpes simplex virus (HSV) vectors, and baculovirus vectors. More specifically, examples of adenovirus vectors for use in the present invention include at least GC44, GC45, GC46, Ad14, Ad35, Ad41, Ad28, Ad26, and Ad5. Examples of non-viral vectors for use in the present invention include plasmids, lipid-based vectors, polymer-based vectors, peptide-based vectors, nanoparticles, and *Sleeping Beauty* transposons. In certain embodiments, the vector may include sequences for serine recombinase mediated integration (e.g., for an *aatP* or *attB* site). Where the vector is a plasmid, mini-circle DNA, or a nanoplasmid, the plasmid, mini-circle DNA or nanoplasmid can further include a bacterial origin of replication, for example one from a ColE1 plasmid.

[0230] An example of a non-viral vector for use in delivering a deoxyribonucleic acid or ribonucleic acid of the present invention is a lipid formulation. Any lipid formulation known in the art for delivering such nucleic acids may be used in the practice of the present invention. In certain embodiments, the nucleic acid can be associated with a lipid. For example, the nucleic acid may encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid.

[0231] Another example of a non-viral vector is a transposon. Any transposon known in the art for delivering a deoxyribonucleic acid or a ribonucleic acid may be used in the practice of the present

invention. When a transposon is used to deliver a nucleic acid, a transposase or a nucleic acid encoding the same is typically also delivered to the cell. A transposase is an enzyme that binds to a transposon and catalyzes its integration into the genome of a cell. In certain embodiments, the vector is a *Sleeping Beauty* transposon. When used, a *Sleeping Beauty* transposase, or a functional fragment or variant thereof, or a nucleic acid encoding the same is also delivered to the cell. Examples of such transposases include, but are not limited to, SB10, SB11, SB100x, and SB110 transposases. *Sleeping Beauty* transposon systems are known in the art and are described for example, in U.S. Patent Nos. 6,489,458 and 8,227,432.

[0232] Any viral vector known in the art for delivering deoxyribonucleic acids or ribonucleic acids may be used in the practice of the present invention. Examples of such vectors include, but are not limited to, adenoviral vectors (*e.g.*, the adenovirus-based Per.C6 system available from Crucell, Inc. (Leiden, The Netherlands)), adeno-associated virus based vectors, lentivirus-based vectors (*e.g.*, the lentiviral-based pLPI from Life Technologies (Carlsbad, Calif.)), retroviral vectors (*e.g.*, the pFB-ERV plus pCFB-EGSH), and herpes virus-based vectors.

[0233] In an embodiment, the viral vector is an adenoviral vector.

Gorilla Adenovirus Vectors

[0234] In preferred embodiments, the adenoviral vector is derived from a gorilla adenovirus, for example, GC44, GC45, or GC46 adenovirus. In some such embodiments, the adenoviral vector is derived from GC44. In other embodiments, the adenoviral vector is derived from GC45.

[0235] In certain embodiments, the adenoviral vector is derived from GC44 and comprises a nucleic acid sequence having at least about 80% sequence identity with a sequence disclosed in U.S. Patent No. 9,233,153 or a functional variant thereof (*e.g.*, a nucleic acid having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with a sequence disclosed in U.S. Patent No. 9,233,153 or a conservatively-substituted variant of a sequence disclosed in U.S. Patent No. 9,233,153).

[0236] In certain embodiments, the adenoviral vector is derived from GC45 and comprises a nucleic acid sequence having at least about 80% sequence identity with a sequence disclosed in U.S. Patent No. 9,629,906 or a functional variant thereof (*e.g.*, a nucleic acid having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with a sequence disclosed in U.S. Patent No. 9,629,906 or a conservatively-substituted variant of a sequence disclosed in U.S. Patent

No. 9,629,906).

[0237] In certain embodiments, the adenoviral vector is derived from GC46 and comprises a nucleic acid sequence having at least about 80% sequence identity with a sequence disclosed in U.S. Patent No. 9,617,560 or a functional variant thereof (*e.g.*, a nucleic acid having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with a sequence disclosed in U.S. Patent No. 9,617,560 or a conservatively-substituted variant of a sequence disclosed in U.S. Patent No. 9,617,560).

[0238] Gorilla adenoviruses are closely related to human adenoviruses, but pre-existing immunity amongst humans for Gorilla adenoviruses (*e.g.*, GC44, GC45, or GC46) is rare and weak. For example, the sero-prevalence of newly-isolated GC44, GC45, or GC46 in humans in the U.S. is 6% compared with 57% for human adenovirus type 5 (Ad5). As such, gorilla adenoviruses retain the advantages of human adenoviruses, including the ability to delete genetic regions to ensure replication incompetence and allow for insertion of transgenes of interest, but are not recognized by human sera from healthy donors. Hollingdale MR, Sedegah M, Limbach K: Development of replication-deficient adenovirus malaria vaccines. *Expert Rev Vaccines* 2017, 16(3):261-271.

[0239] GC44, GC45, and GC46 are newly-isolated and unique gorilla adenovirus strains, isolated from healthy African gorilla stool specimen. Each of these three gorilla adenoviruses are closely related to and clusters phylogenetically with the human species C adenoviruses based on hexon, DNA polymerase and Exon 4 ORF6 protein sequence comparison. Duncan et al., *Virology*, 444:119–123 (2013). The sero-prevalence of each of these three gorilla adenovirus types is less than about 6% in the United States. In comparison, the sero-prevalence of Ad5 type is about 57%, with most of the seropositive individuals having high titers (above 200 IC90). Johnson et al., *Molecular Therapy*, 22:196–205 (2014). Therefore, compared to traditional adenovirus therapies based on the Ad5 serotype, pre-existing neutralizing activity to GC44, GC45, or GC46 is rare and weak in the United States. In addition, comparative studies from human sera samples from Sub-Saharan Africa confirmed the rare and weak pre-existing neutralizing activity in the human population. These data suggest that pre-existing neutralizing activity to GC44, GC45, or GC46 will not significantly interfere with molecular vaccines and therapeutics built on one of these gorilla adenovirus platforms, which makes each of gorilla adenoviruses GC44, GC45, and GC46 well suited as backbone viral vectors.

[0240] In particular, adenovirus vectors with deletions of and/or within the E1 and/or E4 regions may have advantages in terms of safety and efficacy. Gao et al., *Journal of Virology*, 70:8934–8943

(1996). Thus, in certain embodiments, the adenovirus vectors described herein are engineered to delete portions of or the entire E1 and/or E4 regions.

[0241] In certain embodiments, the adenovirus vector is a gorilla adenovirus vector engineered to lack portions of or the entire E1 and/or E4 regions. The deletion in the E1 region may, for example, render the adenovirus vector replication-deficient and include bases 459 through 3411, resulting in deletion of the E1A and E1B promoters and open reading frames. The deletion in the E4 region may, for example, be inclusive of bases 34144 to 36824 and remove all the E4 open reading frames (ORFs), therefore eliminating essential elements for gorilla adenovirus replication. (The gorilla adenovirus coordinates provided herein are based on a wild-type adenovirus genome size of 37,213 base pairs.)

[0242] The modified adenovirus vector backbone lacking portions of or the entire E1 and/or E4 regions provides several advantages. One advantage is that the extended deletions of the adenoviral genome provide enhanced payload capacity to the adenovirus vector. A second advantage is reduced risk of Replication Competent Adenovirus (RCA) generation during adenovirus vector production. A third advantage is that the elimination of E1 and E4 expression products works to further silence other regions of the viral genome.

[0243] Thus, in one aspect described herein, the gorilla adenovirus vectors described herein have the E1 region, or portions thereof, deleted. In another aspect the gorilla adenovirus vectors described herein have the E4 region, or portions thereof, deleted. In another aspect the gorilla adenoviral vectors described herein have both the E1 and E4 regions, or portions thereof, deleted. In one aspect, the deletion(s) in the E1 and/or E4 regions comprise from about 1,500 to about 3,500 base pairs (bp) in length as compared to the wild-type. In another aspect, the deletion(s) in the E1 and/or E4 regions comprise about 3,000 bp in length as compared to the wild-type.

[0244] In certain embodiments, deletion of the E4 region removes all predicted open reading frames (ORFs) therein. To avoid the potential for low levels of production of adenoviral vectors with E4 deletions, spacer sequences may be inserted within the E4 deleted region, to stop any potential transcription initiated from the retained E4 promoter. In one aspect, the gorilla adenoviral vector described herein comprises a spacer sequence inserted in place of the deleted portion of the E4 region. In one aspect, the spacer sequence comprises a Bovine Growth Hormone polyadenylation (BGH polyA) signal sequence that is inserted in place of the deleted E4 ORFs, but any suitable spacer sequence may be used. In some aspects, the spacer sequence is about 10 to about 500 base pairs (bp) in length. For example, the spacer sequence may be about 10, about 20, about 30, about 40, about 50,

about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, about 140, about 150, about 160, about 170, about 180, about 190, about 200, about 210, about 220, about 230, about 240, about 250, about 260, about 270, about 280, about 290, about 300, about 310, about 320, about 330, about 340, about 350, about 360, about 370, about 380, about 390, about 400, about 410, about 420, about 430, about 440, about 450, about 460, about 470, about 480, about 490, or about 500 bp in length. Alternatively, the spacer sequence may be about 50 bp to about 100 bp, about 100 bp to about 150 bp, about 150 bp to about 200 bp, about 200 bp to about 250 bp, about 250 bp to about 300 bp, about 300 bp to about 350 bp, about 350 bp to about 400 bp, about 400 bp to about 450 bp, or about 450 bp to about 500 bp, as compared to the wild-type. The spacer may also be any length within these ranges. For instance, the spacer may be about 250 bp to about 350 bp, about 260 bp to about 340 bp, about 270 bp to about 330 bp, about 280 bp to about 320 bp, or about 290 bp to about 310 bp in length. In one aspect, the spacer sequence is about 300 base pairs in length. In yet another aspect, the spacer sequence is 278 bp.

[0245] In another aspect, the location of the spacer sequence is at about 34,700 thru 35,000 base pairs of the vector genome as compared to the wild-type. In yet another aspect, the location of the spacer sequence is at 34,692 thru 34,969 base pairs of the vector genome as compared to the wild-type.

[0246] In certain embodiments, the vector has all of the E1 and E4 regions deleted. In some such embodiments, the E1 or E4 region is replaced with a genetic construct comprising a transgene or a spacer. In some such embodiments, the E1 region is replaced with the genetic construct. In some embodiments, the E4 region is replaced with the genetic construct. In some embodiments, both the E1 and E4 regions are replaced with the genetic construct. In some embodiments, the E1 region is replaced with the genetic construct and the E4 region is replaced with a spacer.

[0247] In another embodiment, the vector has at least a portion of the E2 and/or E3 region deleted. In certain embodiments, the deletion of at least a portion of the E2 and/or E3 regions increases cloning space and productivity of the vector.

[0248] In one embodiment, the vector has at least a portion of E1, E3, and/or E4 deleted. In certain embodiments, the deletion of at least a portion of the E2 and/or E3 regions increases cloning space and/or productivity of the vector. In some embodiments, the E2 and/or E3 regions are replaced with the genetic construct of the present invention.

[0249] In an embodiment, the viral vector is a lentiviral vector. Vectors derived from retroviruses

such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

[0250] In order to assess the expression of one or more miRNA(s) described herein or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors or non-viral vectors. In other aspects, the selectable marker can be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes can be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neomycin resistance gene (neo) and ampicillin resistance gene and the like. In some embodiments, a truncated epidermal growth factor receptor (HER1t or HER1t-1) tag can be used as a selectable marker gene.

[0251] Reporter genes can be used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tei et al., FEBS Letters 479: 79-82 (2000)). Suitable expression systems are well known and can be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions can be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

VI. Methods for Introducing the miRNA into Cells

[0252] The present invention relates in part to a method for modifying the expression of a gene in a cell, wherein the method comprises introducing the ribonucleic acid of the present invention or the deoxyribonucleic acid of the present invention to the cell. The present invention also relates in part to

the use of a ribonucleic acid of the present invention or the deoxyribonucleic acid of the present invention in the manufacture of a medicament for modifying the expression of a gene.

[0253] The present invention also relates in part to a method for producing a genetically-engineered cell, wherein the method comprises introducing the ribonucleic acid of the present invention or the deoxyribonucleic acid of the present invention to a cell.

[0254] In certain embodiments of the above methods, the method comprises transfecting a cell with the ribonucleic acid of the present invention or the deoxyribonucleic acid of the present invention. In certain embodiments, transfection involves electroporation.

[0255] In certain embodiments, the deoxyribonucleic acid may comprise a transposon, for example a *Sleeping Beauty* transposon. In embodiments wherein a *Sleeping Beauty* transposon is used, a *Sleeping Beauty* transposase or a functional fragment or variant thereof, or a nucleic acid encoding the same, may be introduced to the cell. In certain embodiments wherein the cell is transfected with a transposon, the method further comprises transfecting the cell with a vector encoding a transposase.

[0256] In certain embodiments of the above methods, the cell is transduced with the ribonucleic acid of the present invention or the deoxyribonucleic acid of the present invention. The cells may be transduced with a viral vector comprising such ribonucleic acid or deoxyribonucleic acid.

[0257] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method known in the art. For example, the vector can be transferred into a cell by physical, chemical, or biological means.

[0258] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are known in the art. See, for example, Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (2001)). In some embodiments, a method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection or polyethylenimine (PEI) Transfection. In some embodiments, a method for introduction of a polynucleotide into a host cell is electroporation.

[0259] Chemical methods for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-

based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*e.g.*, an artificial membrane vesicle).

[0260] As a biological method, the ribonucleic acid or the deoxyribonucleic acid of the present invention may, for example, be introduced to the cell using viral-based delivery systems. Representative viral expression vectors include, but are not limited to, the adenovirus-based vectors (*e.g.*, the adenovirus-based Per.C6 system available from Crucell, Inc. (Leiden, The Netherlands)), adeno-associated virus based vectors, lentivirus-based vectors (*e.g.*, the lentiviral-based pLPI from Life Technologies (Carlsbad, Calif.)), retroviral vectors (*e.g.*, the pFB-ERV plus pCFB-EGSH), and herpes virus-based vectors. In an embodiment, the viral vector is a lentivirus vector. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. In general, and in embodiments, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (*e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0261] Regardless of the method used to introduce exogenous nucleic acids into a host cell, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays can be performed. Such assays include, for example, “molecular biological” assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR and “biochemical” assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological means (ELISAs and Western blots).

VIII. Kits and Compositions

[0262] The present invention relates in part to a kit or composition comprising the ribonucleic acid or the deoxyribonucleic acid of the present invention. In certain embodiments, the kit or composition comprises the genetic construct and/or the vector of the present invention. In certain embodiments, the kit comprises a composition of the present invention.

[0263] The present invention also relates in part to a kit or composition as described above for use in modifying the expression of a gene. The present invention further relates in part to a kit or

composition as described above for use in treating a disease or disorder in a subject or for use in the production of a medicament for treating a disease or disorder in a subject.

[0264] In certain embodiments, the kit or composition comprises a transposase.

[0265] In certain embodiments, the kit or composition comprises gene switch components, such as components of the RHEOSWITCH® gene switch components.

[0266] In certain embodiments, the composition further comprises a carrier, a diluent, and/or an excipient. Any carrier, diluent, or excipient known in the art for use with a nucleic acid, vector, or cell is contemplated for use in the practice of the present invention. For example, compositions of the present invention may comprise: buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose, dextrans, or mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives.

[0267] In certain embodiments, the kit comprises a carrier, package, label, container, or instructions for use. Suitable containers include, for example, bottles, vials, syringes, and test tubes.

IX. Methods of Treatment

[0268] Any of the genetic constructs disclosed herein can be used to treat subjects (*e.g.*, humans or other mammals) with diseases or disorders that would benefit from administration of such. In a preferred embodiment, as detailed in the foregoing disclosure, the genetic construct of the present invention comprises at least the following: a (i) tissue-specific promoter (*e.g.*, a liver-specific promoter); (ii) a 5' UTR; (iii) any of the AAT miRNA designs disclosed herein; (iv) silencer/miRNA-resistant AAT(M) transgene; and (v) a 3' UTR. The genetic construct may be delivered to a subject via a vector, preferably a gorilla adenovirus vector.

[0269] Alpha-1 antitrypsin deficiency may result in various diseases, including lung disease or liver disease. In addition, diabetes (types 1 and 2) and cystic fibrosis have been linked to AAT deficiency. *See Kim M, Cai Q, Oh Y. Therapeutic potential of alpha-1 antitrypsin in human disease. Ann Pediatr Endocrinol Metab.* 2018 Sep;23(3):131-135. The genetic construct described herein may be administered to a subject to help treat any one of these diseases linked to AAT deficiency attributed to a pathogenic SERPINA1 allele.

Lung Disease

[0270] Chronic obstructive pulmonary disease (COPD), specifically emphysema and/or

chronic bronchitis, is the most common clinical manifestation of AATD. Bronchiectasis is also associated with AATD. Individuals with severe AATD may manifest the usual signs and symptoms of obstructive lung disease, asthma, and chronic bronchitis (e.g., dyspnea, cough, wheezing, and sputum production). Most individuals with severe AATD have evidence of bronchiectasis on chest CT.

Liver Disease

[0271] The most common manifestation of childhood-onset AATD-associated liver disease is neonatal cholestasis: jaundice, with hyperbilirubinemia and raised serum aminotransferase levels in the early days and months of life. Liver disease in adults (manifesting as cirrhosis and fibrosis) may occur in the absence of a history of neonatal or childhood liver disease. Individuals with AATD are also at risk for hepatocellular carcinoma (HCC). The risk for HCC among individuals with AATD and the PI*ZZ genotype is several times that typically associated with liver cirrhosis. This increased risk has been attributed to failure of apoptosis of injured cells with retained mutant AAT protein, which sends a chronic regeneration signal to hepatocytes with a lesser load of retained mutant AAT protein.

Genotype-Phenotype Correlations

[0272] The risk for lung disease and/or liver disease associated with the following SERPINA1 genotypes is summarized in the table below. Stoller JK et al. Alpha-1 Antitrypsin Deficiency. *Gene Review* (2006). The SERPINA1 alleles in the below table are named with the prefix PI* (protease inhibitor*), serving as an alias for the gene. By way of example the PI*MM genotype indicates that the subject has two copies of the AAT(M) allele and is considered to be homozygous for the AAT(M) allele. The PI*MZ genotype indicates that the subject has one copy of the AAT(M) allele and one copy of the AAT(Z) allele, and is considered to be heterozygous for the AAT(M) and AAT(Z) alleles.

Table 5. SERPINA1 Alleles Genotype-Phenotype Correlations

Genotype	Phenotype
PI*MM	This genotype is associated with a normal serum concentration of AAT and no increased risk of liver or lung disease.
PI*M _{Malton}	Subjects with the M _{Malton} allele are at an increased risk of developing liver disease and/or lung disease.

PI*MZ	In general, nonsmoking individuals with this genotype are not considered to be at increased risk for lung disease; PI*MZ smokers and those with environmental exposures have increased risk of developing COPD
PI*SS	This genotype does not appear to be associated with an increased risk for clinical disease. The S allele is most common among individuals of Iberian descent.
PI*S _{iiyama}	Subjects with the S _{iiyama} allele are at an increased risk of developing liver disease and/or lung disease.
PI*SZ	This genotype is not usually associated with a high risk for liver or lung disease; however, about 11% of individuals with the PI*SZ genotype have serum AAT levels below the protective threshold value (11 µM). Those individuals are at increased risk of developing emphysema with lower zone predominance, as well as chronic bronchitis, especially if they are smokers.
PI*ZZ	Individuals with this genotype have a serum concentration of AAT that is approximately 10%-20% of normal (serum levels of 20-35 mg/dL) and are at high risk for both liver and lung disease. This genotype is present in 95% of affected individuals with clinical manifestations of AATD. Variable disease expressivity in individuals with the PI*ZZ genotype – not accounted for by the presence of known risk factors such as cigarette smoking – suggests the existence of other as-yet unidentified genetic disease modifiers.
PI*FF	While rare, the F allele is associated with AAT that is functionally impaired in binding neutrophil elastase but is quantitatively normal. Individuals with the PI*FF or PI*FZ genotype are deemed to be at increased risk of developing emphysema.
PI*I	Subjects with the I allele, especially when inherited along with the Z allele, are at an increased risk of developing liver disease and/or lung disease.
PI*null-null	Individuals with this genotype have no measurable serum AAT secondary to complete lack of synthesis of AAT. Because protein does not accumulate in the liver, these individuals are not at increased risk of developing liver disease; however, they are at high risk of developing lung disease.

[0273] Type 2 diabetes is a metabolic, chronic inflammatory disease. Diabetic inflammation results in local and systemic insulin resistance, which causes increases in circulating glucose levels. To compensate for increased insulin resistance, the functional β -cell mass first swells to induce hyperinsulinemia. Then, local and systemic inflammatory reactions destroy the resident islet β -cell mass. Blocking the inflammatory pathway can restore the insulin response. The mechanism of action of AAT in type 2 diabetes is currently unclear, but AAT is known to protect pancreatic β -cells from apoptosis by inhibiting caspase-3. Recent studies have shown that the proportion of individuals with low AAT levels (1.0 mg/mL or lower) was 50% higher among diabetic adult patients than the non-diabetic population. The administration of AAT to patients with type 2 diabetes may reduce the severity of the disease.

[0274] Type 1 diabetes, known as juvenile diabetes or insulin-dependent diabetes, is a progressive condition caused by little or no insulin production by the pancreatic islet β -cells. It can be caused by numerous factors, including genetics, infection, or the destruction of the pancreatic islet β -cells by autoreactive T cells. The control of blood glucose levels and the reduction of diabetic complications are critically linked to the protection of pancreatic β -cells. Despite decades of advanced study, the ultimate treatment for this disease has not been determined. However, recent studies on AAT have indicated that controlling inflammation and immune responses aids pancreatic β -cell function through the down-modulation of interleukin (IL)-1 β and other pro-inflammatory cytokines. IL-1 β is known to be harmful to insulin-producing cells. Although the levels of circulating AAT in type 1 diabetic patients may appear to be within normal ranges, AAT function is impaired as a result of extensive non-enzymatic glycation, suggesting that functional AAT levels may play a role in disease progression. In the non-obese diabetic (NOD) mouse model, which is the autoimmune animal model for type 1 diabetes, serum AAT levels are only half those found in the majority of wild type mice. NOD mice recover to normoglycemia 14 days after AAT treatment, and NOD mice that overexpress AAT have reduced insulinitis and do not develop hyperglycemia. In addition, the administration of clinical-grade human AAT to mice with chemically induced diabetes promotes pancreatic islet allograft survival and cytoprotective effects. AAT therapy (80 mg/kg/dose) was reported to be beneficial to β -cell function in adult type 1 diabetic patients. It was also reported that AAT treatment was feasible, without serious adverse complications, and improved glycemic control and serum peak c-peptide levels in pediatric patients with recently diagnosed autoimmune diabetes during a 37-week study period.

[0275] Cystic fibrosis (CF), caused by the mutation of CF transmembrane conductance regulator, progresses from childhood. CF patients have various pulmonary symptoms, such as the production of thick mucus, chronic airway infection, and inflammation, which lead to decreased pulmonary function and early death. In a CF lung, neutrophil counts are elevated over those in a healthy lung and secreted NE leads to the destruction of the defense mechanisms of the lung against infection and inflammation. Thus, in CF, the treatment focus is on decreasing neutrophil hyperactivation and counteracting the effects of NE on the lung. To inhibit NE in the lung during CF progression, early studies have focused on the augmentation of systemic AAT levels by intravenous injection. Recently, a randomized, double-blind, placebo-controlled phase 2a study in CF patients has been further performed to evaluate the safety of 100 or 200 mg of inhaled AAT once daily for 3 weeks in 30 adult subjects and reported that inhalation is safe and well tolerated. Further multiple studies have demonstrated that AAT administered by inhalation can control neutrophil function and NE levels in a dose-dependent fashion as well as inflammation in the lung. However, many obstacles still remain in applying AAT in CF since mixed results have been observed depending on the devices used as well as lung condition and NE concentration of patients.

[0276] In some embodiments, the genetic construct (including a vector comprising the genetic construct) can be used to treat subjects with conditions, diseases or disorders that would benefit from reduction or inhibition of AATD, such as liver disease, lung disease, cystic fibrosis, or diabetes. In some embodiments, the disease is lung disease or liver disease. In further embodiments, the lung disease or liver disease is attributed to alpha-1 antitrypsin deficiency. In some embodiments, the subject is administered a therapeutically effective amount of the genetic construct. Subjects can be adults, adolescents, children or infants. Pharmaceutical compositions comprising the genetic construct can be used to provide methods for therapeutic treatment of diseases such as AATD. Such methods include administration of the pharmaceutical compositions described herein to humans or animals. Further, such methods may be carried out in any manner known in the art, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The nucleic acid, vector, cell, or composition described herein can be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In certain embodiments, intravenous injection may occur via peripheral vein, portal vein, or hepatic artery. In some embodiments, the genetic construct (including any pharmaceutical composition comprising the described genetic construct) is administered once monthly through intravenous injection. In another embodiment, the genetic construct (including any pharmaceutical

composition comprising the described genetic construct) is administered one time through intravenous injection.

[0277] The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices. The appropriate dose can be adjusted accordingly for an adult or a pediatric patient.

[0278] Dosage values may vary with the type and severity of the condition to be alleviated. It is understood that, for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0279] Therapeutic or prophylactic efficacy can be monitored by periodic assessment of treated patients. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens can be useful and are within the scope of the invention. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0280] In some embodiments, the genetic construct described herein is used to treat subjects with AATD, including symptoms, diseases or disorders associated with AATD. AATD liver diseases or disorders include, but are not limited to, chronic hepatitis, cirrhosis, hepatocellular carcinoma, transtonsillitis, cholestasis, fibrosis, and fulminant liver failure. According to some embodiments, the described genetic construct is used to treat at least one symptom in a subject with AATD. Subjects are administered a therapeutically effective amount of the genetic construct.

[0281] In certain embodiments, the invention provides methods of treating a disease or disorder (such as AATD) in a patient in need thereof comprising administering to the patient any genetic construct, vector, or composition described herein.

[0282] In some embodiments, the genetic construct described herein is used to treat or manage clinical symptoms in subjects with AATD liver or lung disease or disorders. The subject is administered a therapeutically effective amount of genetic construct-containing compositions described herein. According to some embodiments, the method comprises administering a

composition comprising the genetic construct described herein to the subject to be treated.

[0283] In certain embodiments, the subject has the AAT(Z) variant. In certain embodiments, the subject has multiple copies (e.g., 2 copies) of the AAT(Z) variant. For example, in certain embodiments, the subject is homozygous for the AAT(Z) variant. In certain embodiments, the subject has the AAT(S) variant. In certain embodiments, the subject has multiple copies (e.g., 2 copies) of the AAT(S) variant. For example, in certain embodiments, the subject is homozygous for the AAT(S) variant. In certain embodiments, the subject has the AAT(F) variant. In certain embodiments, the subject has multiple copies (e.g., 2 copies) of the AAT(F) variant. For example, in certain embodiments, the subject is homozygous for the AAT(F) variant. In certain embodiments, the subject has the AAT(I) variant. In certain embodiments, the subject has multiple copies (e.g., 2 copies) of the AAT(I) variant. For example, in certain embodiments, the subject is homozygous for the AAT(I) variant. In certain embodiments, the subject has an allele associated with intrahepatic inclusions (e.g., Mmalton, Siyama). In certain embodiments, the subject has multiple copies (e.g., 2 copies) of an AAT allele with intrahepatic inclusions. For example, in certain embodiments, the subject is homozygous for an AAT allele with intrahepatic inclusions. In certain embodiments, the subject has multiple AAT allele variants. For example, in certain embodiments, the subject has the AAT(M) variant and the AAT(Z) variant. In certain embodiments, the subject is heterozygous for the AAT(M) variant and the AAT(Z) variant. In certain embodiments, the subject has a pathogenic allele that results in either no mRNA product or no protein production. In certain embodiments, the subject has an AAT serum level of less than about 57 mg/dL.

[0284] In certain embodiments, the genetic construct is used to treat chronic obstructive pulmonary disease, including emphysema and/or chronic bronchitis. In certain embodiments, the genetic construct is used to treat bronchiectasis. In certain embodiments, the genetic construct is used to treat neonatal cholestasis. In certain embodiments, the genetic construct is used to treat cirrhosis. In certain embodiments, the genetic construct is used to treat panniculitis. In certain embodiments, the genetic construct is used to treat granulomatosis with polyangiitis.

Dosing

[0285] The dosing regimen will vary depending on the subject's age, the subject's sex, and the type of active agent to be administered. The dose may be administered hourly, daily, weekly, monthly, or annually. The dose may also be administered as a one-time dose.

[0286] In certain embodiments, the doses are delivered at intervals at least 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, or 31 days apart. In certain embodiments, the doses are delivered at intervals of about twice per day, about once every day, about twice per week, about once every week, about once every two weeks, about once every three weeks, about once every four weeks, or about once every five weeks. In certain embodiments, the second dose is administered about one week after the first dose, about two weeks after the first dose, about three weeks after the first dose, about four weeks after the first dose, or about five weeks after the first dose; the third dose is administered two weeks after the second dose, about three weeks after the second dose, about four weeks after the second dose, about five weeks after the second dose, or about six weeks after the second dose; and the fourth dose is administered about three weeks after the third dose, about four weeks after the third dose, about five weeks after the third dose, about six weeks after the third dose, about seven weeks after the third dose, about eight weeks after the third dose, about nine weeks after the third dose, about ten weeks after the third dose, about eleven weeks after the third dose, or about twelve weeks after the third dose. In one embodiment, the second dose is administered about two weeks after the first dose, the third dose is administered about six weeks after the second dose, and the fourth dose is administered about twelve weeks after the third dose.

[0287] In some embodiments, the dose of the described genetic construct can be: about 200 PU/kg or at least about 300 PU/kg or at least about 400 PU/kg or at least about 500 PU/kg or at least about 600 PU/kg, at least about 700 PU/kg, at least, about 800 PU/kg, at least about 900 PU/kg or at least about 1000 PU/kg, or at least about 1500 PU/kg, or at least about 2000 PU/kg or at least about 2500 PU/kg, or at least about 3000 PU/kg, or at least about 3500 PU/kg, or at least about 4000 PU/kg, or at least about 4500 PU/kg, or at least about 5000 PU/kg.

[0288] In other embodiments, the dose of the described genetic construct can be: about 60,000 PU/kg, or about 70,000 PU/kg, or about 80,000 PU/kg, or about 90,000 PU/kg, or about 100,000 PU/kg, or about 200,000 PU/kg, or about 300,000 PU/kg, or about 400,000 PU/kg, or about 500,000 PU/kg.

[0289] In other embodiments, the dose of the described genetic construct can be from about 1×10^8 PU/kg to about 1×10^{13} PU/kg (e.g., from about 1×10^9 PU/kg to about 1×10^{12} PU/kg, from about 1×10^8 PU/kg to about 1×10^{11} PU/kg or from about 1×10^{10} PU/kg to about 1×10^{13} PU/kg). Ranges can include, but are not limited to, any combination of the lower and upper ranges discussed above

[0290] It should also be understood that the initial dose of the described genetic construct administered may, in some cases, be increased beyond the upper level described above to rapidly

achieve the desired gene expression, protein, and/or mRNA transcript level, or in some cases the initial dose may be less than the optimal value.

[0291] In some embodiments, the dosage amount is contained in a composition having a volume of about 0.1 to about 20 ml, about 0.1 to about 15 ml, about 0.1 to about 10 ml, about 0.1 to about 5 ml, about 0.1 to about 4 ml, about 0.1 to about 3 ml, about 0.1 to about 2 ml, about 0.25 to about 1.75 ml, about 0.5 to about 1.5 ml, about 0.75 to about 1.25 ml, or about 1.0 ml. In some embodiments, the dosage amount is contained in a composition having a volume of about 0.1 ml, about 0.2 ml, about 0.3 ml, about 0.4 ml, about 0.5 ml, about 0.6 ml, about 0.7 ml, about 0.8 ml, about 0.9 ml, about 1.0 ml, about 1.2 ml, about 1.3 ml, about 1.4 ml, about 1.5 ml, about 1.6 ml, about 1.7 ml, about 1.8 ml, about 1.9 ml, about 2.0 ml, about 2.1 ml, about 2.2 ml, about 2.3 ml, about 2.4 ml, about 2.5 ml, about 2.6 ml, about 2.7 ml, about 2.8 ml, about 2.9 ml, about 3.0 ml, about 3.1 ml, about 3.2 ml, about 3.3 ml, about 3.4 ml, about 3.5 ml, about 3.6 ml, about 3.7 ml, about 3.8 ml, about 3.9 ml, about 4.0 ml, about 4.1 ml, about 4.2 ml, about 4.3 ml, about 4.4 ml, about 4.5 ml, about 4.6 ml, about 4.7 ml, about 4.8 ml, about 4.9 ml, or about 5.0 ml.

[0292] In some embodiments, the dosage amount is contained in a composition having a volume of less than about 1 ml, about 1 ml, about 2 ml, about 3 ml, about 4 ml, 5 ml, about 6 ml, about 7 ml, about 8 ml, about 9 ml, about 10 ml, about 11 ml, about 12 ml, about 13 ml, about 14 ml, about 15 ml, about 16 ml, about 17, ml, about 18 ml, about 19 ml, about 20 ml, or more than about 20 ml.

[0293] In some embodiments, the dosage amount is contained in a composition having a volume of less than about 10 ml, about 10 ml, about 15 ml, about 20 ml, about 25 ml, about 30 ml, about 35 ml, about 40 ml, about 45 ml, about 50 ml, about 55 ml, about 60 ml, about 65 ml, about 70 ml, about 75 ml, about 80 ml, about 85 ml, about 90 ml, about 95 ml, about 100 ml, about 105 ml, about 110 ml, about 120 ml, about 130 ml, about 140 ml, about 150 ml, or more than about 150 ml.

[0294] In certain embodiments, the dosage amount in a dose may comprise about 1×10^5 to about 4×10^{14} viral particles, about 1×10^7 to about 1×10^{12} viral particles, about 1×10^8 to about 1×10^{11} viral particles, about 3×10^8 to about 3×10^{11} viral particles, about 1×10^9 to about 1×10^{12} viral particles, about 1×10^9 to about 1×10^{11} viral particles, about 1×10^9 to about 1×10^{10} viral particles, about 1×10^{10} to about 1×10^{12} viral particles, about 1×10^{12} to about 1×10^{14} viral particles, about 2×10^{12} to about 2×10^{14} viral particles, about 1×10^{13} to about 3×10^{13} viral particles, about 3×10^{12} to about 3×10^{14} viral particles, or about 4×10^{12} to about 4×10^{14} viral particles.

[0295] In some embodiments, a dose of the genetic construct may, for example, be about 0.1×10^9 to

about 10×10^{12} viral particles, about 0.5×10^9 to about 9×10^{12} viral particles, about 0.5×10^9 to about 8×10^{12} viral particles, about 0.5×10^9 to about 7×10^{12} viral particles, about 0.5×10^9 to about 6×10^{12} viral particles, about 0.5×10^9 viral particles to about 5×10^{10} viral particles, about 0.1×10^{10} viral particles to about 10×10^{11} viral particles, about 0.5×10^{10} to about 9×10^{11} viral particles, about 0.5×10^{10} to about 8×10^{11} viral particles, about 0.5×10^{10} to about 7×10^{11} viral particles, about 0.5×10^{10} to about 6×10^{11} viral particles, about 0.5×10^{10} viral particles to about 5×10^{11} viral particles, about 0.1×10^{11} to about 10×10^{11} viral particles, about 0.5×10^{11} to about 9.0×10^{11} viral particles, about 0.5×10^{11} to about 8.0×10^{11} viral particles, about 0.5×10^{11} to about 7.0×10^{11} viral particles, or about 0.5×10^{11} to about 6.0×10^{11} viral particles. A dose of the vector may, for example, be about 0.1×10^{11} virus particles, about 0.2×10^{11} virus particles, about 0.3×10^{11} virus particles, about 0.4×10^{11} virus particles, about 0.5×10^{11} virus particles, about 0.6×10^{11} virus particles, about 0.7×10^{11} virus particles, about 0.8×10^{11} virus particles, about 0.9×10^{11} virus particles, about 1.0×10^{11} virus particles, about 0.1×10^{10} virus particles, about 0.2×10^{10} virus particles, about 0.3×10^{10} virus particles, about 0.4×10^{10} virus particles, about 0.5×10^{10} virus particles, about 0.6×10^{10} virus particles, about 0.7×10^{10} virus particles, about 0.8×10^{10} virus particles, about 0.9×10^{10} virus particles, about 1.0×10^{10} virus particles, about 0.1×10^9 virus particles, about 0.2×10^9 virus particles, about 0.3×10^9 virus particles, about 0.4×10^9 virus particles, about 0.5×10^9 virus particles, about 0.6×10^9 virus particles, about 0.7×10^9 virus particles, about 0.8×10^9 virus particles, about 0.9×10^9 virus particles, or about 1.0×10^9 virus particles.

[0296] In certain embodiments, the dosage amount in a dose may comprise about 0.1×10^9 to about 10×10^{11} viral particles, about 0.1×10^9 to about 1.0×10^{11} viral particles, about 0.5×10^9 to about 0.5×10^{11} viral particles, about 0.5×10^9 to about 0.1×10^{11} viral particles, about 1.0×10^{10} to about 10×10^{11} viral particles, about 1.0×10^{10} to about 0.1×10^{11} viral particles, about 0.1×10^{11} to about 10×10^{11} viral particles, about 0.5×10^{11} to about 9×10^{11} viral particles, about 0.5×10^{11} to about 8×10^{11} viral particles, about 0.5×10^{11} to about 7×10^{11} viral particles, about 0.5×10^{11} to about 6×10^{11} viral particles, about 1×10^{10} viral particles, or about 5×10^{10} viral particles.

[0297] In some embodiments, the dosage amount may comprise, for example, about 1×10^6 PU, 2×10^6 PU, 4×10^6 PU, 1×10^7 PU, 2×10^7 PU, 4×10^7 PU, 1×10^8 PU, 2×10^8 PU, 3×10^8 PU, 4×10^8 PU, 1×10^9 PU, 2×10^9 PU, 3×10^9 PU, 4×10^9 PU, 1×10^{10} PU, 2×10^{10} PU, 3×10^{10} PU, 4×10^{10} PU, 1×10^{11} PU, 2×10^{11} PU, 3×10^{11} PU, 4×10^{11} PU, 1×10^{12} PU, 2×10^{12} PU, 3×10^{12} PU, 4×10^{12} PU, 1×10^{13} PU, 2×10^{13} PU, 3×10^{13} PU, 4×10^{13} PU, 1×10^{14} PU, 2×10^{14} PU, 3×10^{14} PU, or 4×10^{14} PU.

[0298] In certain embodiments, the dosage amount may comprise about 1.0×10^5 to about

1.0×10^{10} plaque forming units (PFU), for example, about 0.5×10^5 to about 0.5×10^{10} PFU, about 0.1×10^5 to 0.1×10^{10} PFU, about 1×10^6 to about 1×10^9 PFU, about 0.5×10^6 to about 0.5×10^9 PFU, about 0.1×10^6 to about 0.1×10^9 PFU, about 1×10^7 to about 1×10^8 PFU, about 0.5×10^7 to about 0.5×10^8 PFU, about 0.1×10^7 to about 0.1×10^8 PFU, about 1.0×10^6 to about 1.0×10^9 PFU, about 0.5×10^6 to about 0.5×10^9 PFU, about 1.0×10^7 to about 1×10^8 PFU, about 1.0×10^6 to about 1.0×10^8 PFU, about 0.5×10^6 to about 0.5×10^8 PFU, or about 0.1×10^6 to about 0.1×10^8 PFU.

[0299] In some embodiments, the viral vector may be quantified by Quantitative PCR Analysis (Q-PCR) or analytical HPLC.

Inhibited AAT(Z) Expression

[0300] In some embodiments, the gene expression level of AAT(Z) and/or level of AAT(Z) mRNA transcripts in a subject to whom a described genetic construct is administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98% relative to the subject prior to being administered the described genetic construct or to a subject not receiving the described genetic construct. The gene expression level of AAT(Z) and/or level of AAT(Z) transcripts in the subject may be reduced in a cell, group of cells, and/or tissue of the subject.

[0301] In some embodiments, the protein level of mutant AAT in a subject to whom a described genetic construct has been administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98% relative to the subject prior to being administered the described genetic construct or to a subject not receiving the genetic construct. The mutant AAT protein level in the subject may be reduced in a cell, group of cells, tissue, blood, and/or other fluid of the subject. A reduction in AAT(Z) gene expression, AAT(Z) mRNA transcripts, or mutant AAT protein levels can be assessed by any methods known in the art.

Enhanced AAT(M) Expression

[0302] In some embodiments, the gene expression level of AAT(M) and/or level of AAT(M) mRNA transcripts in a subject to whom a described genetic construct is administered is higher compared to a subject to whom the described genetic construct is not administered. In some embodiments, the gene expression level of AAT(M) and/or level of AAT(M) mRNA transcripts in a subject to whom a described genetic construct is administered is increased by at least about 5%, 10%, 15%, 20%, 25%,

30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98% relative to the subject prior to being administered the described genetic construct or to a subject not receiving the described genetic construct. The gene expression level of AAT(M) and/or level of AAT(M) transcripts in the subject may be increased in a cell, group of cells, and/or tissue of the subject.

[0303] In some embodiments, the protein level of wild-type, functional AAT in a subject to whom a described genetic construct has been administered is increased by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98% relative to the subject prior to being administered the described genetic construct or to a subject not receiving the genetic construct. In other embodiments, the protein level of wild-type, functional AAT in a subject to whom a described genetic construct has been administered is increased by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, or greater than 10.0 times that compared to that in a subject prior to being administered the described genetic construct or to a subject not receiving the genetic construct. The wild-type AAT protein level in the subject may be increased in a cell, group of cells, tissue, blood, and/or other fluid of the subject. An increase in AAT(M) gene expression, AAT(M) mRNA transcripts, or wild-type AAT protein levels can be assessed by any methods known in the art.

[0304] In some embodiments, the serum protein level of wild-type, functional AAT in a subject to whom a described genetic construct has been administered is at least 11 μ M (e.g., from about 11 μ M to about 65 μ M, from about 11 μ M to about 53 μ M, or from about 20 μ M to about 53 μ M). In some embodiments, the serum protein level of wild-type, functional AAT in a subject to whom a described genetic construct has been administered is about 11 μ M to about 65 μ M. In some embodiments, the serum protein level of wild-type, functional AAT in a subject to whom a described genetic construct has been administered is about 11 μ M to about 53 μ M. In some embodiments, the serum protein level of wild-type, functional AAT in a subject to whom a described genetic construct has been administered is about 20 μ M to about 53 μ M.

[0305] The present invention also relates in part to the use of the genetic construct and/or vector described herein, or a composition comprising the same, in the manufacture of a medicament for use in treating a disease or disorder in a subject in need thereof. In preferred embodiments, the genetic constructs described herein, or any vector comprising any genetic construct of the present invention,

or a composition comprising such a nucleic acid or vector, are used to treat liver or lung disease associated with alpha-1 antitrypsin deficiency.

Combination Therapy

[0306] In some embodiments, compositions comprising the genetic construct described herein can be administered as a combination therapy with an additional therapeutic agent. Examples of such agents include biologic agents and small molecules, for example an immunosuppressant or an autophagy enhancing agent.

[0307] In some embodiments, the additional agent is an immunosuppressant. Immunosuppressants reduce or suppress the activity of the immune system to prevent or limit an overactive immune system. Exemplary immunosuppressants include, but are not limited to corticosteroids (e.g., prednisone, prednisolone, and budesonide), calcineurin inhibitors (e.g., cyclosporine and tacrolimus), mTOR inhibitors (e.g., sirolimus and everolimus), IMDH inhibitors (e.g., azathioprine, leflunomide, and mycophenolate), and some biologics (e.g., abatacept, adalimumab, and infliximab).

[0308] In some embodiments, the additional agent is an autophagy enhancing agent. Autophagy is an endogenous pathway to maintain cell function by targeting intracellular components such as proteins or organelles for degradation. In general, autophagy operates by way of a mechanism in which cytosolic proteins or organelles are encircled by a double-membrane vesicle called an autophagosome, which then subsequently fuses with a lysosome for degradation of the intracellular component.

[0309] The autophagy enhancing agents disclosed herein are compounds and compositions that enhance the autophagy pathways in hepatocytes leading to the enhanced clearance of Z-AAT protein globules and reduction of AAT(Z) protein agglomerates retained in the ER. Various classes of known autophagy enhancing agents have been observed to have an autophagy enhancing effect on aggregate-prone proteins, such as AAT(Z), in cells or experimental animal models, which are shown in the following table:

Exemplary Autophagy Enhancing Agents			
Drug	Therapeutic Indications(s)	Reported Pathway of Activity With Respect to Autophagy	Demonstrated Autophagy Effect on Aggregate-Prone Proteins
Ezetimibe	cholesterol lowering	Niemann-Pick-Type C1-Like1, mTORC1 complex	Human cell line, human primary hepatocytes, mouse small intestine

Statins	cholesterol lowering	Depletion of GGDP, activation of AMPK	unknown
Carbamazepine	mood stabilizer, trigeminal neuralgia, epilepsy	Inositol pathway	Rat, monkey and human cell lines, PiZ mice
Oxcarbazepine	treatment of partial seizures	Inositol pathway	unknown
Verapamil	angina, arrhythmias including supraventricular tachyarrhythmias, hypertension	Ca ²⁺ channel blocker	Rat cell line, <i>Drosophila</i> , zebrafish
Loperamide	antidiarrheal	Ca ²⁺ channel blocker	Rat cell line, <i>Drosophila</i> , zebrafish
Nimodipine	antihypertensive for reduction in brain bleeding in patients with subarachnoid hemorrhage	Ca ²⁺ channel blocker	Rat cell line, <i>Drosophila</i> , zebrafish
Nitrendipine	hypertension	Ca ²⁺ channel blocker	Rat cell line, <i>Drosophila</i> , zebrafish
Niguldipine	N/A	Ca ²⁺ channel blocker	High-throughput screening (HTS)
Amiodarone	anti-arrhythmia	Ca ²⁺ channel blocker	Rat cell line, <i>Drosophila</i> , zebrafish
Clonidine	hypertension, severe pain, ADHD	G _i protein signaling pathway via adrenergic and imidazoline receptors	Rat cell line, <i>Drosophila</i> , zebrafish
Rilmenidine	hypertension	G _i protein signaling pathway via adrenergic and imidazoline receptors	Rat cell line, <i>Drosophila</i> , zebrafish
Lithium	mood stabilizer, manic depressive disorder, bipolar disorder	Inositol pathway	Rat, monkey and human cell lines
Valproic acid	mood stabilizer	Inositol pathway	HTS, <i>Drosophila</i>
Fluphenazine	mood stabilizer, schizophrenia	Reduction of intracellular Ca ²⁺ , calpain inhibitor	<i>C. elegans</i> , human cells, PiZ mice
Calpastatin	N/A	Calpain inhibitors	HTS, Zebrafish

Pimozide	mood stabilizer, suppression of tics in patients with Tourette's Disorder	Ca ²⁺ channel blocker	HTS
Fluspirilene	mood stabilizer, schizophrenia	Reduction of intracellular Ca ²⁺	HTS
Glyburide	antidiabetic, glycemic control in patients with type 2 diabetes mellitus	Reduction of intracellular Ca ²⁺	PiZ mice
Metformin	antidiabetic	Upregulation of AMPK	unknown
Rapamycin (sirolimus) and "rapalogs"	immunosuppressant	mTORC1 complex	Rat, monkey, and human cells
Minoxidil	hypertension	K ⁺ ATP channel opener	Mammalian, cells, <i>Drosophila</i> , zebrafish
Trehalose	N/A	mTOR-independent pathway	Mammalian cells
Tyrosine kinase inhibitors	anticancer	Inhibition of Akt-mTOR signaling	unknown
Beclin 1 peptide	N/A (evaluated for autophagy-inducing properties)	Beclin-1 complex	Mammalian cells, mice
Expression-inhibiting oligomeric compound that targets the mRNA of an inhibitor of autophagy	N/A	any pathway affecting autophagy	not yet demonstrated
Bile acid derivatives, Ursodeoxycholic acid, Nor-ursodeoxycholic acid	cholestatic liver injury, biliary fibrosis and AATD	unknown	PiZ mice, humans (clinical)

[0310] Treatment with autophagy enhancing agents in combination with the described genetic construct can lead to a greater effect on globule-containing hepatocytes, compared to those only treated with autophagy enhancing agents alone.

[0311] Autophagy enhancing agents are typically divided into the following two categories: (1) agents that act directly or indirectly on the mammalian target of rapamycin (mTOR) signaling pathway complex to induce autophagy, or (2) mTOR-independent mechanisms, (Chu et al., BioMed Research

Intl (Article ID 459823) (2014)), mTOR is a protein kinase responsible for regulating a host of functions including cell proliferation and growth, cell survival, protein synthesis, transcription, and autophagy. Examples of drugs that are believed to act directly or indirectly through the mTOR signaling complex include, for example, rapamycin and rapamycin analogs, PI3K inhibitors, and ezetimibe. Examples of autophagy enhancing agents that act under mTOR-independent mechanisms include agents that reduce intracellular Ca^{2+} to prevent calpain-1-mediated cleavage of the autophagy gene, such as fluspirilene, or compounds that directly or indirectly impact inositol thus acting on the phosphatidylinositol signaling pathway, such as carbamazepine, valproic acid, and lithium. (Chu *et al.*, BioMed Research Intl (Article ID 459823) (2014)).

[0312] Non-limiting examples of autophagy enhancing agents include the following:

Ezetimibe

[0313] Ezetimibe is known as a potent inhibitor of cholesterol absorption in the small intestine. (Garcia-Calvo, PNAS 102(23): 8132-8137 (2005)). Ezetimibe is an active ingredient included the FDA-approved branded drug Zetia®, which is indicated for lowering plasma cholesterol levels. Zetia® (ezetimibe) is a 10 mg tablet that is to be administered once daily.

[0314] With respect to autophagy, ezetimibe and related compounds are believed to activate the autophagy mechanism by inhibiting the cholesterol efflux Niemann-Pick-type C1 like 1 (NPC1L1), which in turn reduces recruitment of mTOR to the lysosome, inhibiting mTORC1 activity. (Yamamura, 2014, Hepatology 59: 1591-1599).

Carbamazepine

[0315] Carbamazepine is an active ingredient included in several FDA-approved branded drug products indicated for the treatment of conditions associated with epilepsy and neuropathic pain. These include Carbatrol® (100; 200 and 300 mg oral extended-release capsules indicated for use as an anticonvulsant); Equetro® (100, 200 and 300 mg oral extended-release capsules indicated for use as a mood stabilizer for the treatment of acute manic or mixed episodes associated with bipolar I disorder); Tegretol® (100 and 200 mg oral tablets and 100 mg/5 mL oral suspension indicated for use as an anticonvulsant and the treatment of pain associated with true trigeminal neuralgia); and Tegretol XR® (100, 200, and 400 mg oral extended-release tablets indicated for use as an anticonvulsant and the treatment of pain associated with true trigeminal neuralgia). The recommended FDA-approved daily doses for carbamazepine range from 10 to 20 mg/kg/day administered twice daily in small

children, up to doses of 1600 mg daily in adults in rare instances.

[0316] Hidvegi *et al.* reported that carbamazepine was able to mediate a reduction in Z-AAT globules in hepatocytes and ameliorated hepatic fibrosis in the PiZ mouse model of AATD. (Hidvegi *et al.* 2010, Science 329: 229-232). Although the exact mechanism by which carbamazepine and related compounds act to reduce hepatic load of Z-AAT protein in hepatocytes is not understood, its mood-stabilizing effects are similar to the drugs lithium and valproic acid and it may operate under a similar mechanism. The mechanism is believed to involve the inhibition of inositol monophosphatase (IMPase), leading to reduced intracellular inositol levels, thereby negatively regulating autophagy. (Chu *et al.*, BioMed Research Intl (Article ID 459823) (2014) at 4). Additionally, related compounds and derivatives of carbamazepine, such as oxcarbazepine and imipramine, may also have an autophagy enhancing effect in hepatocytes.

Fluphenazine

[0317] Fluphenazine is a trifluoromethyl phenothiazine derivative that acts as an antipsychotic medicine, and is indicated for use in the management of patients with schizophrenia. Fluphenazine can be administered in various salt forms, including fluphenazine hydrochloride, fluphenazine decanoate, and fluphenazine enanthate. Fluphenazine hydrochloride was first marketed over 50 years ago under the brand names Permitil® and Prolixin®. Prolixin® Decanoate (fluphenazine decanoate) and Prolixin® Enanthate (fluphenazine enanthate) were also first marketed in the United States several decades ago. At least fluphenazine hydrochloride (2.5 mg/5 mL elixir; 5 mg/mL concentrate; 2.5 mg/mL injection; and 1 mg, 2.5 mg, 5 mg, and 10 mg oral tablets) and fluphenazine decanoate (25 mg/mL injection) are currently approved in the United States.

[0318] The recommended FDA-approved dosage for fluphenazine hydrochloride is typically 2.5 to 10 mg daily, to be divided and given at 6 or 8 hour intervals. Up to 40 mg daily may be necessary in severe patients. When symptoms are controlled, doses as low as 1 mg daily may be used for maintenance therapy. The recommended FDA-approved dosage for long acting fluphenazine decanoate is generally 12.5 to 25 mg/mL as a starting dose, going up to 100 mg/mL in severe cases. Fluphenazine decanoate typically has an onset of action after 24 to 72 hours after administration, and a single 25 mg dose can be effective in controlling schizophrenic behavior for several weeks.

[0319] Li *et al.* reported that fluphenazine was able to mediate a reduction in Z-AAT globules in hepatocytes and ameliorated hepatic fibrosis in the PiZ mouse model of AATD (Li *et al.* 2014, PLOS ONE 9:e87260). Although the exact mechanism by which fluphenazine and related compounds act to

reduce hepatic load of Z-AAT protein in hepatocytes is not fully understood, fluphenazine and other phenothiazine compounds may activate autophagy by modulating cellular calcium.

Pimozide

[0320] Pimozide is an orally active antipsychotic agent of the diphenyl-butylpiperidine series having the ability to blockade dopaminergic receptors and is indicated for the suppression of motor and phonic tics in patients with Tourette's Disorder. Pimozide can be administered in various salt forms, including fluphenazine hydrochloride, fluphenazine decanoate, and fluphenazine enanthate. Pimozide was the active ingredient in the branded product Orap® (1 mg and 2 mg oral tablets). The recommended FDA-approved dosage for pimozide is as low as 0.05 mg/kg daily for children, up to 10 mg/kg daily for adults. Doses greater than 0.2 mg/kg daily or 10 mg daily are not recommended. Pimozide and related compounds may activate autophagy by modulating cellular calcium.

Fluspirilene

[0321] Fluspirilene is a diphenylbutylpiperidine antipsychotic drug that may be used for the treatment of schizophrenia, and was developed and previously marketed by Janssen Pharmaceuticals. Fluspirilene was marketed worldwide under the brand names Imap® and Redeptin®, and was available in 2 mg/mL and 10 mg/mL formulations. Fluspirilene and related compounds may activate autophagy by modulating cellular calcium.

Glyburide

[0322] Glyburide is an oral blood-glucose-lowering drug of the sulfonylurea class, and is indicated as an adjunct therapy to improve glycemic control in adults with type 2 diabetes mellitus. Micronized glyburide is the active ingredient in the branded drug product Glynase® PresTab® (1.5, 3, and 6 mg oral tablets), and in the branded drub product DiaBeta® (1.25, 2.5, and 5 mg oral tablets). Although there is no fixed dosage regimen for Glynase® PresTab®, the suggested starting does is 1.5 to 3 mg administered daily. Daily doses of more than 12 mg of Glynase® PresTab® are not recommended. Similarly, although there is no fixed dosage regimen for DiaBeta®, the suggested starting dose is 2.5 to 5 mg daily, with a usual maintenance dose of 1.25 to 20 mg daily. Daily doses of more than 20 mg of DiaBeta® are not recommended. Glyburide and related compounds may activate autophagy by modulating cellular calcium.

Clonidine

[0323] Clonidine is an imidazoline derivative centrally-acting alpha-agonist hypertensive agent,

known to stimulate alpha-adrenoreceptors in the brain stem resulting in a decrease in heart rate and blood pressure. The alpha₂-adrenergic agonist properties of clonidine have also been shown to treat attention deficit hyperactivity disorder (ADHD). Clonidine is also known as a centrally-acting analgesic and is indicated for severe pain treatment. Clonidine hydrochloride is the active ingredient in several FDA-approved products for the treatment of hypertension, including the branded drug products Catapres® (0.1, 0.2, and 0.3 mg oral tablets for hypertension), Catapres-TTS®-1, Catapres-TTS®-2, and Catapres-TTS®-3 (7-day transdermal patch system releasing 0.1, 0.2, and 0.3 mg per 24 hours). Clonidine hydrochloride is also the active ingredient in the FDA-approved product Kapvay® (0.1 mg and 0.2 mg extended-release tablets), treatment of ADHD. For severe pain, clonidine hydrochloride is the active ingredient in the FDA-approved products Duraclon® (1 mg/10 mL and 5 mg/10 mL for continuous epidural infusion).

[0324] The initial dose of Catapres® is 0.1 mg twice daily, with therapeutic doses commonly ranging from 0.2 mg to 0.6 mg per day in divided doses. Doses of Catapres® as high as 2.4 mg daily have been shown effective, but are rarely employed. The recommended starting dose of Kapvay® is 0.1 mg daily, which may be increased to 0.4 mg daily. The recommended starting dose of Duraclon® is 30 µg/hr, which may be titrated, and is to be diluted in 0.9% sodium chloride for injection, to a final concentration of no more than 100 µg/mL. Clonidine and related compounds may activate autophagy by modulating cAMP levels. Additionally, related compounds and derivatives of clonidine, such as dexmedetomidine, guanfacine, xylazine, and xylometazoline, may also have an autophagy enhancing effect in hepatocytes.

Verapamil

[0325] Verapamil is a calcium ion antagonist or slow-channel blocker that inhibits calcium ion influx. Verapamil hydrochloride has been approved for several indications, including angina, arrhythmias, and hypertension.]

[0326] Verapamil hydrochloride is the active ingredient in several drug products that have been FDA-approved, including the branded drug product Calan® (40 mg, 80 mg, and 120 mg oral tablets; and 5-mg (2 ml) ampules, 5-mg (2 ml) and 10-mg (4 ml) syringes, and 5-mg (2 ml) and 10-mg (4 ml) vials, for intravenous administration), Calan® SR (120 mg, 180 mg, and 240 mg sustained-release caplets) and Covera-HS® (180 mg and 240 mg extended-release tablets). Calan® oral tablets can be initially administered at 40 mg doses three times per day, but doses can be up to 480 mg/daily in divided doses. Calan® SR oral tablets can be initially administered at 180 mg daily, but doses can be

titrated upwards to 240 mg every 12 hours (480 mg total). For intravenous administration of Calan®, the recommended initial dose for adults is 5-10 mg as an intravenous bolus, with 10 mg repeated doses 30 minutes after the initial dose until the response is adequate, and doses as low as 0.1 mg/kg may be effective in children. Covera-HS® extended-release oral tablets can be initially administered at 180 mg daily, but doses can be titrated upwards to 480 mg every evening, and clinical trials tested doses of up to 540 mg administered at bedtime.

[0327] Verapamil hydrochloride is also the active ingredient in the branded drug product Isoptin® (40 mg, 80 mg, and 120 mg oral tablets; and 2.5 mg/mL for intravenous administration). Verapamil hydrochloride is also the active ingredient in Verelan® (120, 180, 240, and 360 mg sustained-release pellet filled capsules) and Verelan® PM (100, 200, and 300 mg extended-release capsules). Verelan® can be administered up to 480 mg daily, and Verelan® PM can be administered up to 400 mg at bedtime. Verapamil and related compounds may activate autophagy by modulating cellular calcium.

Loperamide

[0328] Loperamide hydrochloride is a synthetic oral antidiarrheal. Loperamide hydrochloride is the active ingredient in the branded drug product Imodium® (2 mg capsules), Imodium® A-D (2 mg caplets and 1 mg/7.5 mL liquid), Imodium® A-D EZ Chews (2 mg chewable tablets), and Imodium® A-D for use in Children (1 mg/7.5 mL liquid). The recommended initial dose of Imodium® in adults is 4 mg, followed by 2 mg after each unformed stool, with a daily dose not exceeding 16 mg. For Imodium® A-D, no more than 4 caplets (8 mg) or 60 mL (8 mg) may be administered every 24 hours in adults. Imodium® A-D EZ Chews are to be administered no more than 4 tablets (8 mg) every 24 hours in adults. Loperamide and related compounds may activate autophagy by modulating cellular calcium.

Nimodipine

[0329] Nimodipine is a calcium channel blocker that has been approved for the improvement of neurological outcome by reducing the incidence and severity of ischemic deficits in patients with subarachnoid hemorrhage from ruptured intracranial berry aneurysms.

[0330] Nimodipine is the active ingredient in the branded drug product Nimotop® (30 mg oral capsules). The recommended dose of Nimotop® oral capsules is 60 mg dosed every four hours for 21 consecutive days (or within 96 hours of the hemorrhage ceasing). Nimodipine is also the active ingredient in the branded drug product Nymalize® (60 mg/20 mL oral solution), for which the

recommended dose is 20 mL (60 mg) dosed every four hours for 21 consecutive days (or within 96 hours of the hemorrhage ceasing). Nimodipine and related compounds may activate autophagy by modulating cellular calcium.

Nitrendipine

[0331] Nitrendipine is a calcium channel blocker with marked vasodilatory action that and is recognized as an effective antihypertensive agent. Nitrendipine has been approved in various parts of the world for hypertension and has been known to reduce the cardiotoxicity of cocaine, and has been marketed as 10 mg and 20 mg tablets, with a recommended daily dose not exceeding 40 mg daily. Nitrendipine and related compounds may activate autophagy by modulating cellular calcium.

Amiodarone

[0332] Amiodarone is an anti-arrhythmia drug. Amiodarone hydrochloride is the active ingredient in the FDA-approved drugs Cordarone® (200 mg tablets) and Nextarone® (150 mg/100 mL and 360 mg/200 mL premixed injection for intravenous use), which are indicated for the treatment of life-threatening recurrent ventricular arrhythmias. Cordarone® is recommended to be administered at 800 to 1,600 mg daily as a loading dose for up to three weeks, with 600 to 800 mg daily dose at approximately one month, and a 400 mg daily dose as a usual maintenance dose. Nextarone® is recommended to be administered at approximately 1,000 mg for the first 24 hours, and mean daily doses over 2,100 mg were associated with an increased risk of hypertension in clinical studies. Amiodarone and related compounds may activate autophagy by inhibiting mTORC1 signaling and/or through an mTOR-independent pathway by modulating cellular calcium.

Lithium

[0333] Lithium is known to alter sodium transport in nerve and muscle cells and effect a shift toward intraneuronal metabolism of catecholamines. Lithium has been approved for the treatment of manic episodes of manic depression and bipolar disorder. Lithium carbonate is the active ingredient in several drug products, including Eskalith® (lithium carbonate 300 mg oral capsules), Eskalith CR® (lithium carbonate 450 mg controlled-release tablets), Lithobid® (300 mg extended-release tablets), and Lithonate® (300 mg oral capsules and 300 mg/5 mL oral syrup). For Eskalith® and Eskalith CR®, most patients are reported to be stable on 900 mg daily doses of lithium, but optimal patient response has been reported in doses of 1800 mg daily. The optimal recommended dose for Lithobid® is 1800 mg daily for acute mania, administered as 900 mg in the morning and 900 mg in the evening, and 1200

mg daily for maintenance therapy, administered as 600 mg in the morning and 600 mg in the evening. For lithium carbonate in the 300 mg/5 mL syrup formulation, optimal patient response for acute mania can usually be established with 10 mL, administered 3 times daily. Lithium is believed to act by way of inhibition of inositol monophosphatase, leading to decreased myo-inositol-1,4,5-triphosphate OP3)

Rapamycin

[0334] Rapamycin, also known as sirolimus, is a macrolide produced by bacterium that has immunosuppressant properties in humans. Rapamycin is the active ingredient in the FDA approved product Rapamune® (0.5 mg, 1 mg, and 2 mg oral tablets, and 60 mg/60 mL solution), which is indicated for the prophylaxis of organ rejection in patients receiving renal transplants. Depending on the patients' condition, the recommended initial starting dose is anywhere from as low as 2 mg daily, and a total maximum daily dose should not exceed 40 mg daily. With respect to autophagy, rapamycin is believed to act by way of inhibition of mammalian target of rapamycin (mTOR), a negative regulator of autophagy. Additionally, related compounds and derivatives of rapamycin, such as temsirolimus, everolimus, deforolimus, and ATP-competitive mTOR kinase inhibitors, may also have an autophagy enhancing effect in hepatocytes.

Minoxidil

[0335] Minoxidil is an antihypertensive peripheral vasodilator. Minoxidil is the active ingredient in the drug product Loniten® (2.5 and 10 mg tablets), which is indicated for hypertension. The recommended initial dose for Loniten® is 5 mg daily, which may be increased to 40 mg daily given in single or divided doses, with the maximum recommended daily dose of 100 mg. Minoxidil and related compounds may activate autophagy by modulating cellular calcium.

Beclin1 Peptide

[0336] With respect to autophagy, beclin1 peptide is believed to act by way of interaction with GAPR-1 (aka GLIPR2), a negative regulator of autophagy.

Bile Acid Derivatives, Ursodeoxycholic Acid/Nor-Ursodeoxycholic Acid

[0337] Other compounds that mimic the effect of activation or upregulation of autophagy may also be useful in combination with expression-inhibiting oligomeric compounds in the treatment of AATD and conditions, manifestations, and diseases caused by AATD. For example, ursodeoxycholic acid (UDCA) and other bile acid derivatives, including bile salts such as taurocholate

and glycocholate are increasingly used for the treatment of cholestatic liver diseases. UDCA has been shown to improve clinical status and liver test results in some children with liver disease associated with AATD. (Lykavieris et al. 2008 *Journal of Pediatric Gastroenterology and Nutrition* 47:623-629). Experimental evidence suggests three major mechanisms of action for bile acids: (1) protection of cholangiocytes against cytotoxicity of hydrophobic bile acids, resulting from modulation of the composition of mixed phospholipid-rich micelles, reduction of bile acid cytotoxicity of bile and, possibly, decrease of the concentration of hydrophobic bile acids in the cholangiocytes; (2) stimulation of hepatobiliary secretion, putatively via Ca^{2+} - and protein kinase C- α -dependent mechanisms and/or activation of p38 (MAPK) and extracellular signal-regulated kinases (Erk) resulting in insertion of transporter molecules (e.g., bile salt export pump, BSEP, and conjugate export pump, MRP2) into the canalicular membrane of the hepatocyte and, possibly, activation of inserted carriers; (3) protection of hepatocytes against bile acid-induced apoptosis, involving inhibition of mitochondrial membrane permeability transition (MMPT), and possibly, stimulation of a survival pathway. (Paumgartner and Beuers, 2002 *Hepatology* 36:525-531). A recent study in the PiZ mouse model of AATD demonstrated that the modified bile acid nor-ursodeoxycholic acid (nor-UDCA) reduced apoptotic signaling and reduced accumulation of mutant Z-AAT in hepatocytes; and these effects from nor-UDCA were associated with an increase in hepatic autophagy. (Tang et al., 2016 *American Journal of Physiology—Gastrointestinal and Liver Physiology* 311:G156-G165). Any agent which improves hepatocyte health as measured by, for example, ALT, AST, and GGT, such as the bile acid derivative disclosed herein, could allow for hepatocytes to more readily clear Z-AAT polymers and improve longer term outcomes in AATD compared to administration with solely AAT expression-inhibiting oligonucleotide compounds. While not intending to be bound by any theory, it is believed that bile acid derivatives may operate at least in part through the mechanism of autophagy.

[0338] The autophagy enhancing agents expressly listed herein and identified in the above table are merely exemplary and are not intended to be limiting to the scope of the application. Any suitable autophagy enhancing agent capable of reducing or eliminating the Z-AAT globules beyond what can be achieved through monotherapy treatment of an AAT expression-inhibiting oligomeric compound may be used. Further, various derivative compounds and analogs to the compounds expressly described herein have similar and related properties, which may allow them to act by the same or similar mechanisms with respect to autophagy or other pathways that lead to benefits in the treatment of AATD. Such compounds are envisioned to fall within the scope of the invention.

[0339] The autophagy enhancing agents disclosed herein can be provided in compositions comprising a pharmaceutically acceptable carrier or pharmaceutically acceptable excipient (including, e.g., vehicles, carriers, and/or diluents). Excipients may include, but are not limited to: absorption enhancers, anti-adherents, anti-foaming agents, anti-oxidants, binders, buffering agents, carriers, coating agents, colors, delivery enhancers, dextran, dextrose, diluents, disintegrants, emulsifiers, extenders, fillers, flavors, glidants, humectants, lubricants, oils, polymers, preservatives, saline, salts, solvents, sugars, suspending agents, sustained release matrices, sweeteners, thickening agents, tonicity agents, vehicles, water-repelling agents, and wetting agents. A pharmaceutically acceptable excipient may or may not be an inert substance.

[0340] The pharmaceutical compositions can contain other additional components commonly found in pharmaceutical compositions. The pharmaceutically-active materials may include, but are not limited to: anti-pruritics, astringents, local anesthetics, or anti-inflammatory agents (e.g., antihistamine, diphenhydramine, etc.). The autophagy enhancing agents may be in the form of any suitable dosage form known in the art for the particular autophagy agent. For example, the autophagy enhancing agents disclosed herein may be administered orally in capsule or tablet form, or any other suitable unit dosage form, in association with the administration of the AAT expression-inhibiting oligomeric compound. Of course, other types of administration of both medicaments, as suitable for the specific autophagy agent selected, are contemplated, such as by nasal spray, by a buccal or sublingual administration dosage form, transdermally, parenterally, by suppository, by sustained release dosage form, etc. Any form of administration will work so long as the proper dosages are delivered.

[0341] In some embodiments, the pharmaceutical compositions comprising the autophagy enhancing agent may further comprise the described genetic construct. In some embodiments, the pharmaceutical compositions comprising the autophagy enhancing agent is separate from the pharmaceutical composition comprising the AAT expression-inhibiting oligomeric compound. The described pharmaceutical compositions can be used to treat a subject having a disease or disorder that would benefit from reduction or inhibition in AAT expression. The described pharmaceutical compositions can be used to treat a subject at risk of developing a disease or disorder that would benefit from reduction or inhibition in AAT expression and Z-AAT globule formation. Diseases and/or disorders that would benefit from such reduction or inhibition may be selected from the list comprising: AATD, chronic hepatitis, cirrhosis, hepatocellular carcinoma, and fulminant hepatic failure. Preferably, the subject is a mammal, most preferably a human patient.

EXAMPLES

[0342] These Examples are provided for illustrative purposes only and are not intended to limit the scope of the invention as set forth in the claims.

[0343] The following table includes abbreviations and special terms that apply to the Examples only. These abbreviations and special terms are not otherwise limiting, and neither replace nor narrow the broader definitions set forth above, which shall continue to apply to the claims.

Table 6: Abbreviations and Special Terms for Use in the Examples.

Abbreviation of Special Term	Explanation
Artificial miRNA	A non-naturally occurring miRNA designed to target a specific gene
miRNA	microRNA, a small RNA species generally 21-23 nucleotides in length that is complementary to a target mRNA transcript and reduces expression of the target transcript through either RNA degradation or inhibition of translation
Pri-miRNA	Primary miRNA, which refers to the initial transcript produced during the transcription of an miRNA-encoding gene
Pre-miRNA	Precursor miRNA, which is the intermediate miRNA species that has undergone the first processing step to remove the RNA stem-loop structure from pri-miRNA. Pre-miRNA undergoes a second processing step in which the mature miRNA is cleaved and loaded into the RNA-induced silencing complex (RISC)
Guide miRNA	Guide miRNA refers to the antisense strand of miRNA that acts as an RNA-induced silencing complex to perform its inhibitory function on its target mRNA.
Passenger miRNA	Passenger miRNA refers to the sense strand of miRNA

	(complementary to the guide miRNA strand) and preferably degrades and does not play a significant role in gene regulation
5'	5' refers to the end of the DNA or RNA strand that has the fifth carbon in the sugar-ring of the deoxyribose or ribose at its terminus
3'	3' refers to the terminating end of the DNA or RNA strand that has the hydroxyl group of the third carbon in the sugar-ring of the deoxyribose or ribose
UTR	An untranslated region, which refers to a section of mRNA that is not translated into protein

Example 1. miRNA Designs for Reduced Expression of AAT(Z)

[0344] This example describes the design of artificial primary miRNAs and artificial mature miRNAs which are the subject of the present invention. Artificial primary miRNA structures were designed to reduce expression of the mutant gene encoding the mutant AAT(Z) variant with the transgene expressing the wild-type AAT(M) variant. The artificial primary miRNA structures were designed so that the polynucleotide sequence encoding at least one of the miRNA structures was sufficiently different from the polynucleotide structure encoding at least one other primary miRNA structure in order to reduce or prevent vector recombinations and/or to reduce or prevent these primary miRNAs from pairing or binding with each of the other. The primary miRNAs were further designed so that the stem-loop secondary structures of each primary miRNA were sufficiently similar to the predicted secondary structures of naturally-occurring miRNAs to reduce or prevent cellular RNAi-based anti-pathogen toxicity. The primary miRNAs were further designed to each comprise mature miRNA sequences for binding different regions of RNA transcripts produced from the same naturally-occurring AAT gene.

Example 2: Design of an Engineered GC44 Vector Encoding Dual miRNA Designs

[0345] This example describes the design of a gorilla adenovirus vector (GC44) encoding two primary miRNA designs (*see* Example 1) of the present invention.

[0346] A GC44 shuttle plasmid was created with a full gene construct built in the shuttle plasmid.

Standard cloning techniques, including restriction digestion and Gibson assembly methods, were performed to generate the shuttle plasmid. The gene construct, comprising two miRNA designs, along with miRNA-resistant wild-type AAT gene (AAT(M) variant), a liver-specific promoter, 5'UTR, and 3'UTR was inserted into the E1 region of the GC44 adenovector in reverse orientation. The liver-specific promoter restricted transgene expression to the liver and the wild-type AAT gene (AAT(M) variant) was modified to have silent mutations sufficient to evade reduction of expression by miRNA.

Example 3: Design of an Engineered GC44 Vector Encoding Triplet miRNA Designs

[0347] This example describes the design of a vector encoding three primary miRNA designs (see Example 1) of the present invention. A GC44 shuttle plasmid is created with a full gene construct built in the shuttle plasmid. Standard cloning techniques, including restriction digestion and Gibson assembly methods, are performed to generate the shuttle plasmid. The gene construct, comprising three miRNA designs, along with miRNA-resistant wild-type AAT gene (AAT(M) variant), a liver-specific promoter, 5'UTR, and 3'UTR, is inserted into the E1 region of the GC44 adenovector in reverse orientation. The liver-specific promoter restricted transgene expression to the liver and the wild-type AAT gene (AAT(M) variant) is modified to have silent mutations sufficient to evade reduction of expression by miRNA.

Example 4: Design of an Engineered GC45 Vector Encoding Dual miRNA Designs

[0348] This example describes the design of a vector encoding two primary miRNA designs (see Example 1) of the present invention. A GC45 shuttle plasmid was created with a full gene construct built in the shuttle plasmid. Standard cloning techniques, including restriction digestion and Gibson assembly methods, were performed to generate the shuttle plasmid. The gene construct, comprising two miRNA designs, along with miRNA-resistant wild-type AAT gene (AAT(M) variant), a liver-specific promoter, 5'UTR, and 3'UTR was inserted into the E1 region of the GC45 adenovector in reverse orientation. The liver-specific promoter restricted transgene expression to the liver and the wild-type AAT gene (AAT(M) variant) was modified to have silent mutations sufficient to evade reduction of expression by miRNA.

Example 5: Design of an Engineered GC45 Vector Encoding Triplet miRNA Designs

[0349] This example describes the design of a vector encoding three primary miRNA designs (see Example 1) of the present invention. A GC45 shuttle plasmid is created with a full gene construct built in the shuttle plasmid. Standard cloning techniques, including restriction digestion and Gibson

assembly methods, are performed to generate the shuttle plasmid. The gene construct, comprising three miRNA designs, along with miRNA-resistant wild-type AAT gene (AAT(M) variant), a liver-specific promoter, 5'UTR, and 3'UTR is inserted into the E1 region of the GC45 adenovector in reverse orientation. The liver-specific promoter restricted transgene expression to the liver and the wild-type AAT gene (AAT(M) variant) is modified to have silent mutations sufficient to evade reduction of expression by miRNA.

Example 6: *In Vivo* Assessment of Liver-Specific Transcript Expression in Mice

[0350] The strength of a CMV promoter and a liver specific promoter were assessed using IVIS® imaging. BALB/C and NSG-PiZ mice were treated with I.V. administration 1×10^{11} PU of a vector with either the CMV promoter or the liver-specific promoter (LSP) driving expression of firefly luciferase (CMV:fLUC or LSP:fLUC) as the transgene construct. NSG-PiZ mice express the mutant human AAT(Z) allele of SERPINA1 and are a model for AATD liver disease.

[0351] IVIS images of mice were taken one day post-administration showed that the liver-specific promoter produced targeted expression of the transgene in liver tissue (FIGs. 1A-1C). When the luminescence was quantified, it was found that the liver-specific promoter achieved similar levels of transgene expression using approximately 10 times less vector as compared to when expression was driven by the CMV promoter (FIGs. 2A-2B).

[0352] In one experiment in which NSG-PiZ mice were treated with a dual miRNA construct containing miR204 and miR206, PAS-D staining showed that liver globules were reduced 28 days after administration (FIGs. 3A-3B and 4A-4B). In addition, the expression of adenovirus DNA from GC44 and GC45 adenovectors in various tissues of NSG-PiZ mice was determined. The number of copies of the adenovector per cell was determined in brain, heart, kidney, lung, liver, ovary, and spleen of NSG-PiZ mice administered 1×10^{11} PU dose of RA-1276 (Group 3), RA-1330 (Group 4), or RA-1331 (Group 5) by IV administration, or NSG (Group 1) or NSG-PiZ mice administered FFB (Group 2) administered an equivalent volume of final formulation buffer (FFB) as a control. RA-1276 is a GC44 vector with the transgene cassette containing a liver-specific promoter driving expression of two AAT(Z)-targeted miRNAs plus the AAT(M) miRNA-resistant transgene inserted in the deleted E1 region in reverse orientation. RA-1330 is a similar vector to RA-1276, but has a CpG-reduced transgene cassette (promoter through 3'UTR). RA-1331 is the transgene cassette from RA-1330 in a GC45 adenovector. Adenovirus DNA was found in all tissues, with the most present in liver and lung (Fig. 5). Expression of the AAT(M) transcript was highest in liver (Fig. 6).

Example 7: *In Vivo* Dose-Dependent Expression of AAT(M) in Mice Treated with Dual miRNA Designs

[0353] This example describes how mice treated with the dual miRNA designs of the present invention demonstrated dose-dependent expression of the AAT(M) variant. C57BL/6 mice, as well as NSG mice, were dosed with a GC44 gorilla adenovirus vector engineered to have a liver-specific promoter and to encode the dual miRNA designs and the miRNA-resistant AAT(M) variant.

[0354] Specifically, C57BL/6 mice were treated with the dual miRNA designs at the following dosage levels systematically administered by a single intravenous injection: 1×10^{11} PU, 5×10^{10} PU, and 2×10^{10} PU. Serum samples from these mice were collected 2, 9, and 17 days post-administration and the expression of hAAT(M) was measured in each sample (*see* FIG. 7A). It was determined that the expression hAAT(M) in the C57BL/6 mice was dose-dependent from a single administration of the vector comprising the AAT(M) variant. The 1×10^{11} PU and 5×10^{10} PU doses were associated with AAT serum concentrations above the $11 \mu\text{M}$ threshold predicted to be therapeutically relevant.

[0355] In addition, NSG mice were treated with 1×10^{11} PU of the dual miRNA designs via a single intravenous injection. Serum samples were collected 2, 9, 16, 23, 30, 44, 58, 72, 79, 93, 107, 121, 128, 142, 156, 170, and 184 days post-administration and the expression of hAAT(M) was measured in each sample (*see* FIG. 7B). It was determined that a single administration of 1×10^{11} PU of the vector comprising the AAT(M) variant resulted in long term expression of hAAT(M) in the serum of NSG mice, with values exceeding the $11 \mu\text{M}$ threshold of predicted therapeutic benefit.

Example 8: *In Vivo* Tolerance and Expression of AAT(M) in Mice Treated with Dual miRNA Designs

[0356] To determine expression of AAT(M) from RA-1276 (FIG. 8A), an *in vitro* experiment using HepG2 cells with the SERPINA1 gene knocked out was performed in which CG dinucleotides were mutated in the promoter, 5' UTR, and transgene where possible. Eight CpG remained in the promoter that were within conserved transcription factor binding regions. The amount of serum AAT provided by CpG-reduced RA-1276 (FIG. 8A).

[0357] The amount of serum AAT provided by a CpG-reduced GC44 adenovector was assessed in immune-competent mice (C57BL/6/J mice). Specifically, a single dose of 1×10^{11} PU/mouse of the GC44 AATD vector (RA-1276) or the CpG-reduced transgene cassette version (RA-1330) was administered intravenously to the mice. Treatment did not negatively impact animal body weight throughout the study compared to the control mice that received an equivalent volume of the final

formulation buffer (FFB) (FIG. 8B). Both adenovectors also provide high levels of AAT(M) expression in the serum well above the 11 μ M threshold of predicted therapeutic benefit (FIG. 9).

[0358] Tolerance to GC44 and GC45 adenovectors was determined. NSG-PiZ mice were administered 1×10^{11} PU of RA-1276 (Group 3), RA-1330 (Group 4), or RA-1331 (Group 5) by IV administration. NSG (Group 1) and NSG-PiZ mice administered FFB (Group 2) were administered an equivalent volume of final formulation buffer (FFB) as controls. RA-1276 is a GC44 vector with the transgene cassette containing a liver-specific promoter driving expression of two AAT(Z)-targeted miRNAs plus the AAT(M) miRNA-resistant transgene inserted in the deleted E1 region in reverse orientation. RA-1330 is a similar vector to RA-1276, but has a CpG-reduced transgene cassette (promoter through 3'UTR). RA-1331 is the transgene cassette from RA-1330 in a GC45 adenovector. NSG-PiZ mice receiving adenovector (groups 3-5) gained weight similarly to control mice (groups 1-2), indicating that the vectors were well tolerated (FIG. 10).

Example 9: *In Vivo* Dose Response Reduction of AAT(Z) in NSG-PiZ Mice Treated with Dual miRNA Designs

[0359] This example describes how NSG-PiZ mice treated with the dual miRNA designs demonstrated reduced expression of the AAT(Z) variant. NSG-PiZ mice were dosed with a GC44 gorilla adenovirus vector engineered to have a liver-specific promoter and to encode the dual miRNA designs and the miRNA-resistant AAT(M) variant.

[0360] Specifically, NSG-PiZ mice were treated with the dual miRNA designs at the following dosage levels systematically administered by a single injection: 1×10^{11} PU, 5×10^{10} , 1×10^{10} , 5×10^9 , and 1×10^9 PU. Liver cells were biopsied from the mice 30 days post-administration and these biopsied liver cells were stained with PAS-D to identify mutant AAT(Z) globule formation (*see* FIG. 11). Accordingly, it was determined that, following treatment with the dual miRNA designs, AAT(Z) globule formation was reduced relative to the dosage level of the dual miRNAs and that the expression of the dual miRNAs was functioning to reduce liver pathology.

[0361] Two additional adenovirus vector constructs also showed efficacy in NSG-PiZ mice. RA-1276 is a GC44 vector with the transgene cassette containing a liver-specific promoter driving expression of two AAT(Z)-targeted miRNAs and a AAT(M) miRNA-resistant transgene inserted in the deleted E1 region in reverse orientation. RA-1330 is a similar vector to RA-1276 but has a CpG-reduced transgene cassette (promoter through 3'UTR). RA-1331 is the transgene cassette from RA-1330 in a GC45 adenovector. NSG-PiZ mice were administered 1×10^{11} PU dose of indicated

adenovector by IV administration, or an equivalent volume of final formulation buffer (FFB) as a control. On day 28 post-administration, liver tissue was harvested and processed for PAS-D staining to visualize the presence of globules, which are a hallmark of aggregated AAT(Z) protein. RNA was extracted from liver tissue on day 28 post-administration and used as input for RT-qPCR to detect AAT Z-allele transcript and the miRNA-resistant AAT(M) transgene transcript. DNA was extracted from liver tissue on day 28 post-administration and used to analyze the number of copies of adenovirus DNA per cell by qPCR. Treatment with RA-1276, RA-1330, and RA-1331 resulted in reduction of liver globules (Fig. 12A) and reduction in AAT(Z) allele transcript expression (Fig. 12B), and an increase in AAT(M) allele transcript expression (Fig. 12C). The presence of vector in liver tissue was confirmed following treatment with RA-1276, RA-1330, and RA-1331 (Fig. 12D).

Example 10: Treatment of Liver Disease Using GC44-Dual miRNA Designs in Patients with AAT(Z)

[0362] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(Z) globule formation, reduced liver pathology and enhanced expression of AAT(M).

Example 11: Treatment of Liver Disease Using GC45-Dual miRNA Designs in Patients with AAT(Z)

[0363] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC45 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(Z) globule formation, reduced liver pathology, and enhanced expression of AAT(M).

Example 12: Treatment of Liver Disease Using GC46-Dual miRNA Designs in Patients with AAT(Z)

[0364] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC46 gorilla adenovirus vector engineered

to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(Z) globule formation, reduced liver pathology, and enhanced expression of AAT(M).

Example 13: Treatment of Liver Disease Using GC44-Triplet miRNA Designs in Patients with AAT(Z)

[0365] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, triplet miRNA designs with at least one miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(Z) globule formation, reduced liver pathology, and enhanced expression of AAT(M).

Example 14: Treatment of Liver Disease Using GC45-Triplet miRNA Designs in Patients with AAT(Z)

[0366] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC45 gorilla adenovirus vector engineered to encode a liver-specific promoter, triplet miRNA designs with at least one miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(Z) globule formation, reduced liver pathology, and enhanced expression of AAT(M).

Example 15: Treatment of Liver Disease Using GC46-Triplet miRNA Designs in Patients with AAT(Z)

[0367] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC46 gorilla adenovirus vector engineered to encode a liver-specific promoter, triplet miRNA designs with at least one miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(Z) globule formation, reduced liver pathology, and enhanced expression of AAT(M).

Example 16: Treatment of Lung Disease Using GC44-Dual miRNA Designs in Patients with AAT(Z)

[0368] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 17: Treatment of Lung Disease Using GC45-Dual miRNA Designs in Patients with AAT(Z)

[0369] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC45 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 18: Treatment of Lung Disease Using GC46-Dual miRNA Designs in Patients with AAT(Z)

[0370] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC46 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 19: Treatment of Lung Disease Using GC44-Triplet miRNA Designs in Patients with AAT(Z)

[0371] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, triplet miRNA designs with at least one miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 20: Treatment of Lung Disease Using GC45-Triplet miRNA Designs in Patients with AAT(Z)

[0372] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC45 gorilla adenovirus vector engineered encode a liver-specific promoter, triplet miRNA designs with at least one miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 21: Treatment of Lung Disease Using GC46-Triplet miRNA Designs in Patients with AAT(Z)

[0373] A patient who has AAT alleles containing a deficient Z-type AAT variant (“AAT(Z)”) is administered a pharmaceutical composition comprising a GC46 gorilla adenovirus vector engineered encode a liver-specific promoter, triplet miRNA designs with at least one miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(Z), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 22: Treatment of Lung Disease Using GC44-miRNA Designs in Patients with AAT(M_{Malton})

[0374] A patient who has AAT alleles containing a deficient M_{Malton}-type AAT variant (“AAT(M_{Malton})”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, at least two miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(M_{Malton}), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 23: Treatment of Liver Disease Using GC44-miRNA Designs in Patients with AAT(M_{Malton})

[0375] A patient who has AAT alleles containing a deficient M_{Malton}-type AAT variant (“AAT(M_{Malton})”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(M_{Malton}), and

an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(M_{Malton}) globule formation, reduced liver pathology, and enhanced expression of AAT(M).

Example 24: Treatment of Lung Disease Using GC44-miRNA Designs in Patients with AAT(Siiyama)

[0376] A patient who has AAT alleles containing a deficient Siiyama-type AAT variant (“AAT(Siiyama)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, at least two miRNA designs with at least miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(Siiyama), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 25: Treatment of Liver Disease Using GC44-miRNA Designs in Patients with AAT(Siiyama)

[0377] A patient who has AAT alleles containing a deficient Siiyama-type AAT variant (“AAT(Siiyama)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(Siiyama), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(Siiyama) globule formation, reduced liver pathology, and enhanced expression of AAT(M).

Example 26: Treatment of Lung Disease Using GC44-miRNA Designs in Patients with AAT(F)

[0378] A patient who has AAT alleles containing a deficient F-type AAT variant (“AAT(F)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, at least two miRNA designs with at least miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(F), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 27: Treatment of Liver Disease Using GC44-miRNA Designs in Patients with AAT(F)

[0379] A patient who has AAT alleles containing a deficient F-type AAT variant (“AAT(F)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(F), and an miRNA-resistant

AAT(M) variant. Following administration, the patient demonstrates reduced AAT(F) globule formation, reduced liver pathology, and enhanced expression of AAT(M).

Example 28: Treatment of Lung Disease Using GC44-miRNA Designs in Patients with AAT(I)

[0380] A patient who has AAT alleles containing a deficient I-type AAT variant (“AAT(I)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, at least two miRNA designs with at least miRNA targeting any AAT allele disclosed herein and at least a second miRNA targeting AAT(I), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced lung pathology and enhanced expression of AAT(M).

Example 29: Treatment of Liver Disease Using GC44-miRNA Designs in Patients with AAT(I)

[0381] A patient who has AAT alleles containing a deficient I-type AAT variant (“AAT(I)”) is administered a pharmaceutical composition comprising a GC44 gorilla adenovirus vector engineered to encode a liver-specific promoter, dual miRNA designs with one miRNA targeting any AAT allele disclosed herein and a second miRNA targeting AAT(I), and an miRNA-resistant AAT(M) variant. Following administration, the patient demonstrates reduced AAT(I) globule formation, reduced liver pathology and enhanced expression of AAT(M).

Example 30. *In Vivo* Efficacy of GC44-dual miRNA Designs in a NSG-PiZ mouse model.

[0382] The efficacy of a series of vectors were tested. Vectors and study design are described in FIG. 13. Mice with expression of miRNAs targeting AAT had fewer liver globules (FIGs. 14A-14C). Adenovector DNA was detected at the highest abundance in the liver (FIG. 15). AAT(M) transgene RNA expression was strongest in the liver (FIG. 16A-16B) and mature miRNA was detected in the liver (FIG. 17A-17B).

SEQUENCE LISTING

Nucleic Acid Sequences Encoding Backbone pri-miRNA Sequences			
Synthetic miRNA	DNA encoding 5' backbone sequence	DNA encoding stem loop	DNA encoding 3' backbone sequence
miR204	AGGAGGGTGGGGG TGGAGGCAAGCAG AGGACTTCCTGATC GCGTACCCATGGCT ACAGTCTTTCTTCA TGTGACTCGTGGAC (SEQ ID NO: 1)	GAGAATATATGAAGG (SEQ ID NO: 2)	GTTCAATTGTCATCA CTGGCATCTTTTTTG ATCATTGCACCATCA TCAAATGCATTGGG ATAACCATGAC (SEQ ID NO: 3)
miR206	GATGCTACAAGTG GCCCCACTTCTGAGA TGCGGGCTGCTTCT GGATGACACTGCTT CCCGAGG (SEQ ID NO: 4)	TATGGATTACTTTGCT A (SEQ ID NO: 5)	TTTCGGCAAGTGCCT CCTCGCTGGCCCCAG GGTACCACCCGGAG CACAGGTTTGGTGAC CTT (SEQ ID NO: 6)
miR17	TTAGCAGGAAAAA AGAGAACATCACC TTGTAAACTGAA GATTGTGACCAGTC AGAATAATGT (SEQ ID NO: 7)	TGATATGTGCAT (SEQ ID NO: 8)	GCATTATGGTGACA GCTGCCTCGGGAAG CCAAGTTGGGCTTTA AAGTGCAGGGCCTG CTGATGT (SEQ ID NO: 9)
miR150	AGGGACTGGGCCC ACGGGGAGGCAGC GTCCCCGAGGCAG CAGCGGCAGCGGC	CTGGGCTCAG (SEQ ID NO: 11)	CAGGGACCTGGGGA CCCCGGCACCCGCA GGCCCCAAGGGGTG AGGTGAGCGGGCAT

	GGCTCCTCTCCCCA TGGCCCTG (SEQ ID NO: 10)		TGGGACCTCCCCTCC CTGTACTC (SEQ ID NO: 12)
miR150	AGGGACTGGGCCC ACGGGGAGGCAGC GTCCCCGAGGCAG CAGCGGCAGCGGC GGCTCCTCTCCCCA TGGCC (SEQ ID NO: 13)	TGCTGGGCTCAGACC (SEQ ID NO: 14)	GGACCTGGGGACCC CGGCACCGGCAGGC CCCAAGGGGTGAGG TGAGCGGGCATTGG GACCTCCCCTCCCTG TACTC (SEQ ID NO: 15)
miR16	CTTCTGAAGAAAA TATATTTCTTTTTA TTCATAGCTCTTAT GATAGCAATGTCA GCAGTGCCT (SEQ ID NO: 16)	TTAAGATTCTAAAATT ATCT (SEQ ID NO: 17)	AAGTAAGGTTGACC ATACTCTACAGTTGT GTTTTAATGTATATT AATGTTACTAATGTG TTTT (SEQ ID NO: 18)
miR30a	CAGGTTAACCCAA CAGAAGGCTAAAG AAGGTATATTGCTG TTGACAGTGAGCG AC (SEQ ID NO: 19)	CTGTGAAGCCACAGA TGGG (SEQ ID NO: 20)	TGCCTACTGCCTCGG ACTTCAAGGGGCTA CTTTAGGAGCAATTA TCTTGTTTA (SEQ ID NO: 21)
miR126	CCGGGGTCCTGTCT GCATCCAGCGCAG CATTCTGGAAGAC GCCACGCCTCCGCT GGCGACGG (SEQ ID NO: 22)	CTGTGACACTCAAAC (SEQ ID NO: 23)	CCGTCCACGGCACC GCATCGAAAACGCC GCTGAGACCTCAGC CTTGACCTCCCTCAG CGTGG (SEQ ID NO: 24)

miR122	CAATGGTGGAATG TGGAGGTGAAGTT AACACCTTCGTGGC TACAGAGTTTCCTT AGCAGAGCTG (SEQ ID NO: 25)	TGTCTAAACTAT (SEQ ID NO: 26)	TAGCTACTGCTAGGC AATCCTTCCCTCGAT AAATGTCTTGGCATC GTTTGCTTTGAGCAA GAAGGTTTCTCTGAT ATCAGTCTTCTCAAT CT (SEQ ID NO: 27)
miR214	ACAGGCTGATTGT ATCTGTCTATGAGC AAAGGAAACCTGA AGGAACCAAGGGC CTGGCTGGACAGA GTTGTCATGTG (SEQ ID NO: 28)	GCAGAACATCCGCTC ACCTGT (SEQ ID NO: 29)	CACATGACAACCCA GCCTGAATGACAAC CAGCCATTGAAAGA AAGCAGCCCTCACA CCATAGCATCTA (SEQ ID NO: 30)
miR214	ACAGGCTGATTGT ATCTGTCTATGAGC AAAGGAAACCTGA AGGAACCAAGGGC CTGGCTGGACAGA GTTGTCATGTGTC (SEQ ID NO: 31)	AGAACATCCGCTCACC T (SEQ ID NO: 32)	CACATGACAACCCA GCCTGAATGACAAC CAGCCATTGAAAGA AAGCAGCCCTCACA CCATAGCATCTA (SEQ ID NO: 33)
miR29b1	GGGTTTATTGTAAG AGAGCATTATGAA GAAAAAATAGAT CATAAAGCTTCTTC AGG (SEQ ID NO: 34)	GATTTAAATAGTGATT GTC (SEQ ID NO: 35)	CTTGGGGGAGACCA GCTGCGCTGCACTAC CAACAGCAAAAGAA GTGAATGGGACAGC T (SEQ ID NO: 36)

miR29b1	GGGTTTATTGTAAG AGAGCATTATGAA GAAAAAATAGAT CATAAAGCTTCTTC AGGAA (SEQ ID NO: 37)	TTTAAATAGTGATTG (SEQ ID NO: 38)	GTTCTTGGGGGAGA CCAGCTGCGCTGCAC TACCAACAGCAAAA GAAGTGAATGGGAC AGCT (SEQ ID NO: 39)
miR133a1	TTTACCAATGAAA AGCATTTAACTGTT TTGGATTCCAACT AGCAGCACTACAA TGCTTTGCTA (SEQ ID NO: 40)	TCGCCTCTTCAATGGA (SEQ ID NO: 41)	TAGCTATGCATTGAT TACTACGGGACAAC CAACGTTTTTCATTG TGAATATCAATTACT TGCCA (SEQ ID NO: 42)
miR26a1	TGAAGCCACAGGA GCCAAGAGCAGGA GGACCAAGGCCCT GGCGAAGGCCGTG GCCTCG (SEQ ID NO: 43)	GTGCAGGTCCCAATG (SEQ ID NO: 44)	CGGGGACGCGGGCC TGGACGCCGGCATC CGGGCTCAGGACCC CCCTCTCTGCCAGAG GC (SEQ ID NO: 45)
miR412	GTCTTGGAGGCTG GGGCACCTCGGGG AAGGACGCCGGCA TCAGCACCATTCTG GGGTACGGGGATG GA (SEQ ID NO: 46)	GTTTCT	GCCGTCCGTATCCGC TGCAGCCTGTGGGG CCTGCGGGCCGGGG AGCCGATCGCGCTTC AGCTCAGCGCCT (SEQ ID NO: 48)
miR-19	CCAATAATTCAAG CCAAGCAAGTATA TAGGTGTTTAAATA GTTTTTGTTTGCAG	TACAAGAAGAATGTA GT (SEQ ID NO: 50)	TGGTGGCCTGCTATT TCCTTCAAATGAATG ATTTTACTAATTTT GTGTACTTTTATTGT

	TCCTCTG (SEQ ID NO: 49)		G (SEQ ID NO: 51)
miR-21	TGTCTGCTTGTTTT GCCTACCATCGTGA CATCTCCATGGCTG TACCACCTTGTCGG G (SEQ ID NO: 52)	CTGTTGAATCTCATGG (SEQ ID NO: 53)	TCTGACATTTTGGA TCTTTCATCTGACCA TCCATATCCAATGTT CTCATTTAAACA (SEQ ID NO: 54)
miR-142	GGAGTCAGGAGGC CTGGGCAGCCTGA AGAGTACACGCCG ACGGACAGACAGA CAGTGCAGTCACC (SEQ ID NO: 55)	AACAGCACTGGAGGG (SEQ ID NO: 56)	GATGAGTGTACTGTG GGCTTCGGAGATCA CGCCACTGCTGCCGC CCGCTGCCCCGCCACC ATCTTC (SEQ ID NO: 57)
miR-494	CAGTTCTGTTTTGA TTTTTTTTGTTTGT TTTTGATCAGTGCT AATCTTCGATACTC GAAGGA (SEQ ID NO: 58)	TCTTTATTTATGA (SEQ ID NO: 59)	TTTTTTAGTATCAAA TCCCACCCTGGAGGC ACTTCCTGTTCTGA TGCAGCCTTCAGGG AGG (SEQ ID NO: 60)
miR-1915	CGGACCACGGTGT CCCCTTCTCTCCAG CTGGGGGTCTCGG GTCCTGGCGCTGA GAGGCCGC (SEQ ID NO: 61)	GTGCACCCGTG (SEQ ID NO: 62)	GCGGCCCTAGCGAC CTGCGGCGGCGCCG GGAAAGCCCTGCCT CTGCAGCGGGTCCC AGGGGTC (SEQ ID NO: 63)
miR-22	CATTTTCCCTCCCT TTCCCTTAGGAGCC TGTTCTCTCACGC	TATGTCCTGACCCAGC TA	TGCCCTCTGCCCCTG GCTTCGAGGAGGAG GAGGAGCTGCTTCC

	CCTCACCTGGCTGA GCCGCA (SEQ ID NO: 338)	(SEQ ID NO: 339)	CCATCATCTGGAAG GTG (SEQ ID NO: 340)
miR-26a1	ATGAAGCCACAGG AGCCAAGAGCAGG AGGACCAAGGCCC TGGCGAAGGCCGT GGCCTCG (SEQ ID NO: 341)	GTGCAGGTCCCAATG (SEQ ID NO: 342)	CGGGGACGCGGGCC TGGACGCCGGCATC CGGGCTCAGGACCC CCCTCTCTGCCAGAG GC (SEQ ID NO: 343)
miR-486	TGTGGTGCTGGGG GCTTCAGCGGCCG GCTCTGATCTCCAT CCTCCCTGGGGCA (SEQ ID NO: 344)	CCCTTCATGCTGC (SEQ ID NO: 345)	ACTCGGGGTGGGAG TCAGCAGGAGGTGA GGGGGCATGGTGGC CCCAGTGCAGC (SEQ ID NO: 346)
miR181a	TCTCCCATCCCCTT CAGATACTTACAG ATACTGTAAAGTG AGTAGAATTCTGA GTTTTGAGGTTGCT TCAGTG (SEQ ID NO: 597)	TTGGAATTAAAATCAA A (SEQ ID NO: 598)	CTATGGCTAACCATC ATCTACTCCATGGTG CTCAGAATTCGCTGA AGACAGGAAACCAA AGGTGGACACACCA GG (SEQ ID NO: 599)

Splice donor and splice acceptor site sequences

5' side of intron (SEQ ID NO: 291)	AGGTAAGAGTCGATCG (SEQ ID NO: 291)
3' side of intron (SEQ ID NO: 292)	ACGCGTTACTAACTGGTACCTCTTTTTTTTTTTTGATATCCTGCAGGC (SEQ ID NO: 292)

Exemplary control sequences

miRNA Target	SEQ ID NO	miRNA backbone	DNA sequence of Pri-miRNA
Scrambled control 2	582	miR206, shorter arms	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGC TGCTTCTGGATGACACTGCTTCCCGAGGCATTTC GCCCTATCTGCAAGTACTATGGATTACTTTGCTA GTGGTGTAGATAGGGTGAAATGTTTCGGCAAGTG CCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCA CAGGTTTGGTGACCTT
neg. ctl c.elegans cel-miR-67	583	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCG AGGCAGCAGCGGCAGCGGCGGCTCCTCTCCCAT GGCCCTGTCACAACCTCCTAGAAAGAGTACTGGG CTCAGACCCCTTTTCAGGCCGTTGTGACAGGGAC CTGGGGACCCCGGCACCGGCAGGCCCAAGGGG TGAGGTGAGCGGGCATTGGGACCTCCCCTCCCTG TACTC
neg. ctl c.elegans cel-miR-67	584	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTT CCTGATCGCGTACCCATGGCTACAGTCTTTCTTCA TGTGACTCGTGGA CTTGTACTACACAAAAGTACT GTGAGAATATATGAAGGACAGGCTTTAGTGTA GT ATGCGTTCAATTGTCATCACTGGCATCTTTTTTGA TCATTGCACCATCATCAAATGCATTGGGATAACC ATGAC
210bp stuffer	585	-	GGTAAGTCATGACTCCCTACAATGGACATGATCA TAGTAGGTACTATAAGGCACCTAGCTATACCCTC CTATAGAGAGTTTGAGTCTATTGTAGCAAGTCTT TCTTTTAGGGCCTAGGACTCTTCCCACTCTCTTCC ATCGCCTAAGCTCTAACTCTTCCTTGTAGTAGAA ATAAGTCACTTCTAAGGCTGGGACCCCTCCTAGAC CCTAA

Scrambled control 1	586	miR206	TTAGGATGAGTTGAGATCCCAGTGATCTTCTCGC TAAGAGTTTCCTGCCTGGGCAAGGAGGAAAGAT GCTACAAGTGGCCCACTTCTGAGATGCGGGCTGC TTCTGGATGACACTGCTTCCCGAGGCATTTCGCC CTATCTGCAAGTACTATGGATTACTTTGCTAGTG GTGTAGATAGGGTGAAATGTTTCGGCAAGTGCCT CCTCGCTGGCCCCAGGGTACCACCCGGAGCACAG GTTTGGTGACCTTCTTCCTCATCAGGGCTTTGTGC CAGCAAATGACTCCCTCACCAAGGAAGCAAGAG CCTCTGAATCCCATCTGGGCTCTTCCTGAACACCC CTATCTCCCCCTCT
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Sequences from GC44 AATD Adenovector		
Element	Nucleotide Sequence	SEQUENCE ID NO.
Liver promoter	CCCCAGGTAAATTTTAAAAAGCAGTCAAAAG TCCAAGTGGCCCTTGGCAGCATTTACTCTCTCT GTTTGCTCTGGTTAATAATCTCAGGAGCACAA ACATTCTGGAGGCAGGAGAAGAAATCAACAT CCTGGACTTATCCTCTGGGCCTCTCCCCACCTT CGATGGCCCCAGGTAAATTTTAAAAAGCAGT CAAAAGTCCAAGTGGCCCTTGGCAGCATTTAC TCTCTCTGTTTGCTCTGGTTAATAATCTCAGGA GCACAAACATTCTGGAGGCAGGAGAAGAAAT CAACATCCTGGACTTATCCTCTGGGCCTCTCCC CACCTTCGAACTAGCCACTAGCCTGAGGCTGG TCAAAATTGAACCTCCTCCTGCTCTGAGCAGCC TAGGGGGCAGACTAAGCAGAGGGCTGTGCAG ACCCACATAAAGAGCCTACTGTGTGCCAGGCA CTTCACCCGAGGCACTTCACAAGCATGCTTGG GAATGAAACTTCCAACCTTTGGGATGCAGGT GAAACAGTTCCTGGTTCAGAGAGGTGAAGCGG	587

	CCTGCCTGAGGCAGCACAGCTCTTCTTTACAGA TGTGCTTCCCCACCTCTACCCTGTCTCACGGCC CCCCATGCCAGCCTGACGGTTGTGTCTGCCTCA GTCATGCTCCATTTTTCCATCGGGACCATCAAG AGGGTGTGTTGTGTCTAAGGCTGACTGGGTAAC TTTGGATGAGCGGTCTCTCCGCTCCGAGCCTGT TTCCTCATCTGTCAAACGGGCTCTAACCCACTC TGATCTCCCAGGGCGGCAGTAAGTCTTCAGCA TCAGGCATTTTGGGGTGACTCAGTAAATGGTA GATCTTGCTACCAGTGGAACAGCCACTAAGGA TTCTGCAGTGAGAGCAGAGGGCCAGCTAAGTG GTA CTCTCCCAGAGACTGTCTGACTCACGCCAC CCCCTCCACCTTGGACACAGGACGCTGTGGTTT CTGAGCCAGGTACAATGACTCCTTTTCGGTAAG TGCAGTGGAAGCTGTACACTGCCCAGGCAAAG CGTCCGGGCAGCGTAGGCGGGCGACTCAGATC CCAGCCAGTGGACTTAGCCCCTGTTTGCTCCTC CGATAACTGGGGTGACCTTGGTTAATATTCACC AGCAGCCTCCCCCGTTGCCCCTCTGGATCCACT GCTTAAATACGGACGAGGACAGGGCCCTGTCT CCTCAGCTTCAGGCACCACCACTGACCTGGGA CAG	
GAPDH 5'UTR	AAATTGAGCCCGCAGCCTCCCGCTTCGCTCTCT GCTCCTCCTGTTTCGACAGTCAGCCGCATCTTCT TTTGCCTCGCCAGCCGAGCCACATCGCTCAGA CACC	588
Dual AAT miRNA-encoding sequence	AGGTAAGAGTCGATCGAGGAGGGTGGGGGTG GAGGCAAGCAGAGGACTTCCTGATCGCGTACC CATGGCTACAGTCTTTCTTCATGTGACTCGTGG ACACGCTCTTCAGATCATAGGCCTGAGAATAT ATGAAGGAGGCTGTGAAGTGAAGAGCGCGTTC	589

	AATTGTCATCACTGGCATCTTTTTTGATCATTG CACCATCATCAAATGCATTGGGATAACCATGA CGATGCTACAAGTGGCCCACTTCTGAGATGCG GGCTGCTTCTGGATGACACTGCTTCCCGAGGCC ATGTTGACCGTCGACATTGAATATGGATTACTT TGCTATTCTTGTCGATGGTCAGCATGGTTTCGG CAAGTGCCTCCTCGCTGGCCCCAGGGTACCAC CCGGAGCACAGGTTTGGTGACCTTACGCGTTA CTAACTGGTACCTCTTTTTTTTTTTTGATATCCT GCAGGC	
AAT with silent mutations to avoid silencing by the AAT miRNAs	ATGCCTAGCTCCGTGTCCTGGGGAATCCTCCTG CTCGCCGGACTGTGTTGCCTCGTGCCTGTGTCC CTGGCTGAGGACCCTCAAGGCGATGCCGCTCA GAAAACCGATACCTCACACCATGACCAAGACC ATCCACATTCAATAAGATTACCCCTAACCTCG CCGAGTTTGCTTTCTCCCTGTATAGACAACTGG CTCACCAAAGCAATAGCACAAACATTTTCTTTA GCCCTGTGTCCATCGCTACCGCTTTCGCTATGT TAAGCCTCGGCACAAAGGCTGACACACACGAT GAGATTCTGGAAGGCCTCAACTTTAACCTCAC CGAAATCCCTGAGGCTCAGATTCACGAAGGCT TTCAGGAACTGCTCAGAACACTGAATCAGCCT GACTCCCAGCTCCAGCTCACCACAGGCAATGG CCTCTTCCTCAGCGAAGGCCTCAAGCTCGTTGA CAAGTTTCTGGAAGATGTAAAGAACTGTATC ACTCCGAGGCTTTCACAGTGAATTTTCGGAGAC ACAGAGGAAGCCAAAAAGCAAATTAATGACT ATGTGGAGAAAGGAACGCAAGGCAAAATCGT CGATCTCGTGAAAGAATTGGACAGGGACACAG TGTTTGCCCTCGTGAATTACATTTTCTTCAAAG GAAAGTGGGAGAGGCCTTTCGAGGTCAAGGAT	590

	ACAGAAGAGGAAGACTTTCACGTAGATCAAGT GACAACCGTCAAGGTCCCCATGATGAAAAGAC TCGGCATGTTCAATATCCAACACTGTAAGAAA CTGTCCAGCTGGGTGCTCCTGATGAAGTATTTA GGCAACGCTACCGCTATCTTTTTCCTCCCCGAT GAGGGCAAATTGCAGCATCTGGAAAACGAACT GACACACGATATCATTACCAAATTCCTTAGAAA ATGAGGATAGAAGGAGCGCCTCTCTCCACCTC CCCAAGCTGTCCATCACAGGCACATACGATCT CAAATCTGTCTTGGGACAGCTCGGCATTACCA AAGTGTTTAGCAATGGCGCTGACCTCAGCGGA GTGACAGAGGAAGCCCCCTCTGAAACTGTCCAA GGCTGTGCATAAGGCTGTGCTCACCATTGATG AAAAAGGTACAGAAGCCGCTGGCGCTATGTTT CTGGAAGCCATTCCCATGAGCATTCCCCCTGA GGTCAAGTTTAACAAACCGTTCGTGTTTCTGAT GATCGAACAGAATACCAAAAGCCCTCTGTTTA TGGGCAAAGTCGTGAATCCCACACAGAAATGA	
hGH+ ACTb terminator 3'UTR	TGATGGGTGGCATCCCTGTGACCCCTCCCCAGT GCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGT GCCCACCAGCCTTGTCCTAATAAAATTAAGTTG CATCATTTTGTCTGACTAGGTGTCCTTCTATAA TATTATGGGGTGGAGGGGGGTGGTATGGAGCA AGGGGCAAGTTGGGAATTACCCAGAGTGCAGG TGTGTGGAGATCCCTCCTGCCTTGACATTGAGC AGCCTTAGAGGGTGGGGGAGGCTCAGGGGTCA GGTCTCTGTTCCCTGCTTATTGGGGAGTTCCTGG CCTGGCCCTTCTATGTCTCCCCAGGTGCCCCAG TTTTTCTGGGTTACCCAGAGTGCAGATGCTTG AGGAGGTGGGAAGGGACTATTTGGGGGTGTCT GGCTCAGGTGCCATGCCTCACTGGGGCTGGTT	591

	GGCACCTGCATTTCTGGGAGTGGGGCTGTCTC AGGGTAGCTGGGCACGGTGTCCCTTGAGTGG GGGTGTAGTGGGTGTTCTAG	
Full genetic construct cassette inserted in E1	CCCCAGGTTAATTTTTAAAAAGCAGTCAAAAG TCCAAGTGGCCCTTGGCAGCATTTACTCTCTCT GTTTGCTCTGGTTAATAATCTCAGGAGCACAA ACATTCTGGAGGCAGGAGAAGAAATCAACAT CCTGGACTTATCCTCTGGGCCTCTCCCCACCTT CGATGGCCCCAGGTTAATTTTTAAAAAGCAGT CAAAAGTCCAAGTGGCCCTTGGCAGCATTTAC TCTCTCTGTTTGCTCTGGTTAATAATCTCAGGA GCACAAACATTCTGGAGGCAGGAGAAGAAAT CAACATCCTGGACTTATCCTCTGGGCCTCTCCC CACCTTCGAAGTAGCCACTAGCCTGAGGCTGG TCAAAATTGAACCTCCTCCTGCTCTGAGCAGCC TAGGGGGCAGACTAAGCAGAGGGCTGTGCAG ACCCACATAAAGAGCCTACTGTGTGCCAGGCA CTTCACCCGAGGCACTTCACAAGCATGCTTGG GAATGAACTTCCAACCTTTGGGATGCAGGT GAAACAGTTCCTGGTTCAGAGAGGTGAAGCGG CCTGCCTGAGGCAGCACAGCTCTTCTTTACAGA TGTGCTTCCCCACCTCTACCCTGTCTCACGGCC CCCCATGCCAGCCTGACGGTTGTGTCTGCCTCA GTCATGCTCCATTTTTCCATCGGGACCATCAAG AGGGTGTTTGTGTCTAAGGCTGACTGGGTAAC TTTGATGAGCGGTCTCTCCGCTCCGAGCCTGT TTCCTCATCTGTCAAACGGGCTCTAACCCACTC TGATCTCCAGGGCGGCAGTAAGTCTTCAGCA TCAGGCATTTTGGGGTGACTCAGTAAATGGTA GATCTTGCTACCAGTGGAACAGCCACTAAGGA TTCTGCAGTGAGAGCAGAGGGCCAGCTAAGTG	592

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SEQ ID NO. 593: Full sequence of GC44 adenovirus with original AATD transgene cassette
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CCTACGTCACCCGCCCCGCCTCTCCCCGCCACCTCATTATCATATTGGCCACAATCC
AAAATAAGGTATATTATTGATGATG

CpG-Reduced Versions of GC44 AATD Adenovector		
Element	Nucleotide Sequence	SEQUENCE ID NO.
CpG-reduced version of liver promoter	CCCCAGGTTAATTTTAAAAAGCAGTCAAAAGTCCAAGTG GCCCTTGGCAGCATTTACTCTCTCTGTTTGCTCTGGTTAATA ATCTCAGGAGCACAAACATTCTGGAGGCAGGAGAAGAAA TCAACATCCTGGACTTATCCTCTGGGCCTCTCCCCACCTTC AATGGCCCCAGGTTAATTTTAAAAAGCAGTCAAAAGTCC AAGTGGCCCTTGGCAGCATTTACTCTCTCTGTTTGCTCTGGT TAATAATCTCAGGAGCACAAACATTCTGGAGGCAGGAGA AGAAATCAACATCCTGGACTTATCCTCTGGGCCTCTCCCCA CCTTCTAACTAGCCACTAGCCTGAGGCTGGTCAAAATTGAA CCTCCTCCTGCTCTGAGCAGCCTAGGGGGCAGACTAAGCA GAGGGCTGTGCAGACCCACATAAAGAGCCTACTGTGTGCC AGGCACTTCACCAGAGGCACTTCACAATCATGCTTGGGAA TGAAACTTCCAACCTCTTTGGGATGCAGGTGAAACAGTTCCT GGTTCAGAGAGGTGAAGAGGCCTGCCTGAGGCAGCACAGC TCTTCTTTACAGATGTGCTTCCCCACCTCTACCCTGTCTCAC AGCCCCCATGCCAGCCTGACAGTTGTGTCTGCCTCAGTCA TGCTCCATTTTCCATCTGGACCATCAAGAGGGTGTTTGTG TCTAAGGCTGACTGGGTAACCTTTGGATGAGCAGTCTCTCCA CTCAGAGCCTGTTTCCTCATCTGTCAAACCTGGCTCTAACCC ACTCTGATCTCCCAGGGAGGCAGTAAGTCTTCAGCATCAG GCATTTTGGGGTGACTCAGTAAATGGTAGATCTTGCTACCA GTGGAACAGCCACTAAGGATTCTGCAGTGAGAGCAGAGGG CCAGCTAAGTGGTACTCTCCCAGAGACTGTCTGACTCACGC CACCCCTCCACCTTGGACACAGGACACTGTGGTTTCTGAG CCAGGTACAATGACTCCTTTCAGTAAGTGCAGTGGAAGCT	594

	GTACACTGCCCAGGCAAAGCATCCGGGCAGCGTAGGCGGG CGACTCAGATCCCAGCCAGTGGACTTAGCCCCTGTTTGCTC CTCCGATAACTGGGGTGACCTTGGTTAATATTCACCAGCAG CCTCCCCCGTTGCCCCTCTGGATCCACTGCTTAAATACGGA CTAGGACAGGGCCCTGTCTCCTCAGCTTCAGGCACCACCAC TGACCTGGGACAG	
CpG-reduced version of GAPDH 5'UTR	AAATTGAGCCCACAGCCTCCCACCTCACTCTCTGCTCCTCC TGTTCAACAGTCAGCTGCATCTTCTTTTGAGTCTCCAGCAG AGCCACATCTCTCAGACACC	595
CpG-reduced version of AAT transgene resistant to miRNAs	ATGCCTAGCTCTGTCTCCTGGGGAATCCTCCTGCTTGCTGG ACTGTGTTGCTTGGTGCCTGTGTCCCTGGCTGAGGACCCTC AAGGAGATGCTGCACAGAAAACAGATACCTCACACCATGA CCAAGACCATCCCACATTCAATAAGATTACCCCTAACCTTG CTGAGTTTGCTTTCTCCCTGTATAGACAACTGGCTCACCAA AGCAATAGCACAAACATTTTCTTTAGCCCTGTGTCCATAGC AACTGCATTTGCCATGTTAAGCCTGGGCACAAAGGCTGAC ACACATGATGAAATTCTGGAAGGCCTCAACTTTAACCTCAC AGAAATCCCTGAGGCTCAGATTCATGAGGGCTTTCAGGAA CTGCTCAGAACTGAATCAGCCTGACTCCCAGCTCCAGCT CACCACAGGCAATGGCCTCTTCTCTCAGAAGGCCTCAAGT TGGTAGACAAGTTTCTGGAAGATGTAAAGAACTGTATCA CTCTGAGGCCTTCACAGTGAATTTTGAGATACAGAGGAA GCCAAAAAGCAAATTAATGACTATGTGGAGAAAGGAACAC AAGGCAAAATTGTTGACCTTGTTAAAGAATTGGACAGGGA CACAGTGTGTTGCCCTGGTCAATTACATTTTCTTCAAAGGAA AGTGGGAGAGGCCTTTTGAGGTCAAGGATACAGAAGAGGA AGACTTTCATGTTGATCAAGTGACAACTGTCAAGGTCCCCA TGATGAAAAGACTGGGCATGTTCAATATCCAACACTGTAA GAACTGTCCAGCTGGGTGCTCCTGATGAAGTATTTAGGCA ATGCAACTGCTATCTTTTTCTCCAGATGAGGGCAAATTG	596

	CAGCATCTGGAAAATGAACTGACACATGATATCATTACCA AATTCTTAGAAAATGAGGATAGAAGGTCAGCCTCTCTCCA CCTCCCCAAGCTGTCCATCACAGGCACTTATGATCTCAAAT CTGTCCTGGGACAGCTGGGAATTACCAAAGTGTTTAGCAAT GGTGCAGACCTCTCTGGGGTGACAGAGGAAGCCCCCTCTGA AACTGTCCAAGGCTGTGCATAAGGCTGTGCTCACCATTGAT GAAAAAGGTACAGAAGCAGCAGGTGCAATGTTTCTGGAAG CCATTCCCATGAGCATTCCCCCTGAGGTCAAGTTTAACAAA CCCTTTGTCTTTCTGATGATTGAGCAGAATACCAAAAGCCC TCTGTTTATGGGCAAAGTTGTAAATCCCACACAGAAATGA	
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Exemplary AAT(M) Amino Acid Sequence (SEQ ID NO: 600)
MPSSVSWGILLLAGLCCLVPVSLAEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFS LYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQEL LRTLNQPDSQLQLTTGNGLFLSEGLKLVDFKLEDVKKLYHSEAFVNFVGDTEEAQKQIND YVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFVVDTEEEDFHVDQVTTVKV PMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLEN EDRRSASLHLPKLSITGTDLKSVLGQLGITKVFVSGADLSGVTEEAPLKLSKAVHKAVLT IDKGTAAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK

Claims

1. A therapeutic genetic construct encoding: (a) a first pre-miRNA; (b) a second pre-miRNA; and (c) a transgene encoding AAT(M).
2. The genetic construct of claim 1, wherein at least 7 nucleotides separate the nucleic acids encoding the first pre-miRNA and the nucleic acid encoding the second pre-miRNA.
3. The genetic construct of claim 1, wherein the first and second pre-miRNAs each comprise a guide miRNA that inhibits the expression of an allele encoding a mutant variant of alpha-1 antitrypsin (AAT).
4. The genetic construct of claim 3, wherein each guide miRNA independently inhibits the expression of a mutant variant of AAT that is AAT(Z), AAT(S), AAT(I), AAT(Siiyama), or AAT(M_{malton}).
5. The genetic construct of claim 3, wherein each guide miRNA inhibits the expression of AAT(Z).
6. The genetic construct of claim 3, wherein the guide miRNA of the first pre-miRNA is encoded by the nucleic acid of SEQ ID NO: 64, or one that hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 64, and the guide miRNA of the second pre-miRNA is encoded by the nucleic acid of SEQ ID NO: 66, or one that hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 66.
7. The genetic construct of claim 1, wherein the first and second pre-miRNAs each independently comprise backbone segments that are identical to the corresponding backbone segments of miR16, miR17, miR19, miR21, miR22, miR26a1, miR29b1, miR30a, miR122, miR126, miR133a1, miR142, miR150, miR155, miR181a, miR204, miR206, miR214, miR412, miR486, miR494, or miR1915.
8. The genetic construct of claim 1, wherein the first and second pre-miRNAs each independently comprise backbone segments that are identical to the corresponding backbone segments of miR16, miR17, miR21, miR22, miR26a1, miR142, miR150, miR204, or miR206.

9. The genetic construct of claim 1, wherein the first pre-miRNA comprises backbone segments that are identical to the corresponding backbone segments of miR204 and the second miRNA comprises backbone segments that are identical to the corresponding backbone segments of miR206.
10. The genetic construct of claim 1, wherein the first pre-miRNA is encoded by the nucleic acid of SEQ ID NO: 347, or one that hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 347, and the second pre-miRNA is encoded by the nucleic acid of SEQ ID NO: 348 or one that hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 348.
11. The genetic construct of claim 1, wherein the first and second pre-miRNAs are encoded by the nucleic acid of SEQ ID NO: 267 or one that hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 267.
12. The genetic construct of claim 1, wherein the first and second pre-miRNAs are encoded by the nucleic acid of SEQ ID NO: 267.
13. The genetic construct of claim 1, wherein the transgene encoding AAT(M) differs from the wild-type gene encoding AAT(M) by silent mutations that allow it to evade reduction of expression by the pre-miRNAs.
14. The genetic construct of claim 1, wherein the transgene encoding AAT(M) comprises a nucleic acid sequence having at least about 80% sequence identity with SEQ ID NO: 590 or 596.
15. The genetic construct of claim 1, wherein the transgene encoding AAT(M) comprises a nucleic acid sequence having at least about 90% sequence identity with SEQ ID NO: 590 or 596.
16. The genetic construct of claim 1, wherein the transgene encoding AAT(M) comprises a nucleic acid sequence having at least about 95% sequence identity with SEQ ID NO: 590 or 596.
17. The genetic construct of claim 1, wherein the transgene encoding AAT(M) comprises the nucleic acid sequence of SEQ ID NO: 590 or 596 or one that hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 590 or 596.
18. The genetic construct of claim 1, wherein the transgene encoding AAT(M) comprises the nucleic acid sequence of SEQ ID NO: 590 or 596.

19. The genetic construct of claim 1, wherein the transgene encoding AAT(M) comprises the nucleic acid sequence of SEQ ID NO: 590.
20. The genetic construct of claim 1, wherein the transgene encoding AAT(M) encodes an amino acid sequence having at least 80% sequence identity with SEQ ID NO: 600.
21. The genetic construct of claim 1, wherein the transgene encoding AAT(M) encodes an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 600.
22. The genetic construct of claim 1, wherein the transgene encoding AAT(M) encodes an amino acid sequence having at least 95% sequence identity with SEQ ID NO: 600.
23. The genetic construct of claim 1, wherein the transgene encoding AAT(M) encodes the amino acid sequence of SEQ ID NO: 600 or a conservatively-substituted variant thereof.
24. The genetic construct of claim 1, wherein the transgene encoding AAT(M) encodes the amino acid sequence of SEQ ID NO: 600.
25. The genetic construct of claim 1, further comprising a liver-specific promoter.
26. The genetic construct of claim 1, wherein the liver-specific promoter comprises the nucleic acid sequence of SEQ ID NO: 587 or 594.
27. A vector comprising the genetic construct of any one of claims 1–26.
28. The vector of claim 27, wherein the vector is a plasmid, a viral vector, or a non-viral vector.
29. The vector of claim 28, wherein the viral vector is an adenoviral vector.
30. The vector of claim 29, wherein the adenoviral vector is lacking portions of or the entire E1 and/or E4 regions.
31. The vector of claim 29, wherein the adenoviral vector is a gorilla adenoviral vector.
32. The vector of claim 29, wherein the adenoviral vector is a GC44 gorilla adenoviral vector.

33. The vector of claim 27, comprising a nucleic acid sequence having at least 80% sequence identity with SEQ ID NO: 593.
34. The vector of claim 27, comprising a nucleic acid sequence having at least 90% sequence identity with SEQ ID NO: 593.
35. The vector of claim 27, comprising a nucleic acid sequence having at least 95% sequence identity with SEQ ID NO: 593.
36. The vector of claim 27, comprising the nucleic acid sequence of SEQ ID NO: 593 or one that hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 593.
37. The vector of claim 27, comprising the nucleic acid sequence of SEQ ID NO: 593.
38. A method of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the vector of claim 27.
39. The method of claim 38, wherein the disease or disorder is a liver or lung disease associated with alpha-1 antitrypsin deficiency.
40. The method of claim 38, wherein the disease or disorder is COPD, bronchiectasis, neonatal cholestasis, cirrhosis, and fibrosis, or hepatocellular carcinoma.
41. The method of claim 38, wherein the therapeutically effective amount comprises about 1×10^9 and about 1×10^{12} particle units (PU).
42. The method of claim 38, wherein the method further comprises administering an additional therapeutic agent.
43. The method of claim 42, wherein the additional agent is an immunosuppressant.
44. A composition comprising the genetic construct of any one of claims 1–26.
45. The composition of claim 44 for use in treating a disease or disorder in a subject in need thereof.
46. A use of the genetic construct of any one of claims 1–26 in the manufacture of a medicament for use in treating a disease or disorder in a subject in need thereof.
47. A kit comprising the genetic construct of any one of claims 1–26 and instructions for use.